WEST Search History

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☐	L20	L19 and (serum-free medium)	5
☐	L19	L18 and expression	86
☐	L18	L17 and mammalian	86
☐	L17	L16 and (scFv dimer)	105
☐	L16	L15 and linker	4355
☐	L15	diabody or svFv	4778
☐	L14	(kontermann)[IN]	20
☐	L13	wo 200053790	1
☐	L12	wo 2000053790	0
☐	L11	L10 and mammalian	2
☐	L10	L9 and animal	2
☐	L9	L8 and eukaryotic	3
☐	L8	L5 and express	3
☐	L7	L5 and express or expression	510024
☐	L6	L5 and host	0
☐	L5	L4 and dimer and linker	3
☐	L4	L2 and diabody	5
☐	L3	L2 and scFV	4
☐	L2	(kontermann)[IN]	20
☐	L1	(kontermann re)[IN]	0

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Search	Most Recent Queries	Time	Result
#59	Search **muller and kontermann**	15:32:13	14
#58	Search **brusselbach and kontermann**	15:32:05	0
#57	Search **brusselbach and muller and kontermann**	15:31:56	0
#56	Search **brusselbach and muller**	15:31:38	20
#55	Search **brusselbach and korn**	15:31:26	0
#54	Search **kontermann and bispecific** Limits: **Publication Date to 2000/4/17**	15:24:43	6
#49	Search **kontermann diabody** Limits: **Publication Date to 2000/4/17**	15:17:24	4
#50	Search **kontermann and diabody** Limits: **Publication Date to 2000/4/17**	15:16:18	4
#48	Search **konterman diabody** Limits: **Publication Date to 2000/4/17**	15:16:00	0
#47	Search **brusselbach diabody** Limits: **Publication Date to 2000/4/17**	15:15:36	0
#46	Search **brusselbach** Limits: **Publication Date to 2000/4/17**	15:13:52	17
#45	Search **brusselbach korn** Limits: **Publication Date to 2000/4/17**	15:13:43	0
#43	Search **single chain Fv and dimer or diabody and animal and cell** Limits: **Publication Date to 2000/4/17**	14:22:03	36
#41	Search **single chain Fv and dimer or diabody and animal** Limits: **Publication Date to 2000/4/17**	14:21:41	39
#39	Search **single chain Fv and dimer or diabody** Limits: **Publication Date to 2000/4/17**	14:16:50	53
#38	Search Limits: **Publication Date to 2000/4/17**	14:15:07	1283268 2
#28	Search **scFV and diabody** Limits: **Publication Date to 2000/4/17**	14:14:38	12
#1	Search **scFV and dimer** Limits: **Publication Date to 2000/4/17**	13:44:47	23

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NEWS	14	JUl 19	Coverage of Research Disclosure reinstated in DWPI
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NEWS	16	AUG 28	ADISCTI Reloaded and Enhanced
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number of left parentheses.

=> s diabody and linker and ((mammalian or animal) and cell)
L1          35 DIABODY AND LINKER AND ((MAMMALIAN OR ANIMAL) AND CELL)

=> duplicate remove l1
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L2          28 DUPLICATE REMOVE L1 (7 DUPLICATES REMOVED)

=> d L2 bib abs 1-28

L2   ANSWER 1 OF 28  CAPLUS  COPYRIGHT 2006 ACS on STN
AN   2005:1171482  CAPLUS
DN   143:438502
TI   Diabodies specific to Streptococcus surface antigen I/II for
     diagnosis and treatment of oral disease such as periodontitis and dental
     caries
IN   Finnern, Ricarda; Fischer, Rainer
PA   Fraunhofer-Gesellschaft Zur Foerderung der Angewandten Forschung e.v.,
     Germany
SO   PCT Int. Appl., 54 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.          KIND   DATE        APPLICATION NO.         DATE
     ---------------     ----   --------    --------------------    --------
PI   WO 2005103085        A1    20051103    WO 2005-EP4284          20050421
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
             GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ,
             LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA,
             NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL,
             SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA,
             ZM, ZW
         RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM,
             AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,
             EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT,
             RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML,
             MR, NE, SN, TD, TG
PRAI US 2004-564396P      P     20040422
```

AB Common oral diseases such as periodontitis and dental caries can be prevented effectively by passive immunization. The present invention provides human single chain Fv (scFv) and diabody antibody fragments based on the binding characteristics of the murine monoclonal antibody Guy's 13. Like the parent antibody, these derivs. bind specifically to SAI/II, the surface adhesin of Streptococcus and the human diabody derivative is capable of aggregating streptococcal cells, making it a useful candidate therapeutic agent for passive immunization against oral diseases.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 2 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
AN 2004:1035080 CAPLUS
DN 142:1787
TI CD46 as a receptor used by the adenoviruses of subtype B for their cell entry, and modified CD46 fused to a component capable of binding to a cell surface molecules for genetic vector use
IN Beerli, Roger R.; Bachmann, Martin F.
PA Cytos Biotechnology AG, Switz.
SO Eur. Pat. Appl., 52 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 1482052	A1	20041201	EP 2003-11184	20030527
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
WO 2004106532	A1	20041209	WO 2004-EP5762	20040527
WO 2004106532	C1	20050331		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1627067	A1	20060222	EP 2004-739419	20040527
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, FI, RO, CY, TR, BG, CZ, EE, HU, PL, SK				
PRAI EP 2003-11184	A	20030527		
WO 2004-EP5762	W	20040527		

AB The inventors report the identification of CD46 (MCP) as a receptor used by adenoviruses of the subgroup B, such as Ad3 and Ad7, for their cell entry. The invention relates to the use of Ad3 and Ad7-based genetic vectors. The invention provides modified polypeptides comprising two functional components: first, a polypeptide derived from the extracellular region of CD46 as a specific binding site for adenoviruses of the subgroup B, and second, a component capable of binding to a cell surface mol. Such modified polypeptides are able to direct adenovirus-infection specifically to cells having said cell surface mol. on their surface. The invention provides nucleic acid sequences encoding fusion proteins comprising (a) a polypeptide derived from the extracellular domain of CD46 and (b) a heterologous polypeptide, methods for the production of the modified polypeptides and suitable recombinant expression vectors and host cells. Pharmaceutical compns. comprising the modified polypeptide of the invention are useful together with recombinant, genetically

engineered adenovirus of subtype B for the treatment and prophylaxis of disorders and diseases, like cancer.

RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 3 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2004:857634 CAPLUS
DN 141:348840
TI Anti-CD22 diabodies for treating hematopoietic malignancies such as lymphoma and leukemia
IN Tsuchiya, Masayuki; Kimura, Naoki; Fukuda, Tatsuya
PA Chugai Seiyaku Kabushiki Kaisha, Japan
SO PCT Int. Appl., 77 pp.
CODEN: PIXXD2
DT Patent
LA Japanese
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2004087763	A1	20041014	WO 2004-JP4696	20040331
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1609803	A1	20051228	EP 2004-724770	20040331
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL, SK				
PRAI JP 2003-96950	A	20030331		
WO 2004-JP4696	W	20040331		

AB Two anti-CD22 antibodies having been published, CD22 diabodies in which variable regions of the heavy chain and the light chain are bonded together via a 5mer linker are constructed. The 2 diabodies, LL2 and RFB4, are examined in binding to lymphoma cells and activity of inducing cell death (apoptosis). As a result, it is found out that both of these diabodies bind to a Raji cell (i.e., a B lymphoma cell line) and have an activity of inducing apoptosis in the Raji cell and a Daudi cell which is also a B lymphoma cell line. These results indicate that degraded antibodies recognizing CD22 are usable as apoptosis inducers in tumor cells such as lymphocyte cells.

RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 4 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2004:252106 CAPLUS
DN 140:269512
TI Multivalent and multispecific engineered antibodies
IN Holliger, Kaspar-philipp; Griffiths, Andrew David; Hoogenboom, Hendricus Renerus J. M.; Malmqvist, Magnus; Marks, James David; McGuinness, Brian Timothy; Pope, Anthony Richard; Prospero, Terence Derek; Winter, Gregory Paul
PA Medical Research Council, UK
SO U.S. Pat. Appl. Publ., 98 pp.
CODEN: USXXCO
DT Patent
LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004058400	A1	20040325	US 2002-247839	20020920
PRAI	US 2002-247839		20020920		

AB The authors disclose antibody constructs comprising a first heavy chain variable region and a second light chain variable region, the domains being linked but incapable of associating with each other to form an antigen binding site. These constructs associate to form antigen binding multimers, such as dimers, which may be multivalent or have multispecificity. The domains may be linked by a short peptide linker or may be joined directly together. Bispecific dimers may have longer linkers. Methods of preparation of the polypeptides and multimers and diverse repertoires thereof, and their display on the surface of bacteriophage for easy selection of binders of interest, are disclosed, along with many utilities.

L2 ANSWER 5 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2004-13881 BIOTECHDS
TI Novel human chimeric or complementarity determining region transplant antibody specific for prostrate gland specific membrane antigen, useful as diagnostic and therapeutic agent of cancer;
vector-mediated gene transfer and expression in host cell for recombinant antibody production
PA KYOWA HAKKO KOGYO KK
PI JP 2004000045 8 Jan 2004
AI JP 2002-158727 31 May 2002
PRAI JP 2002-158727 31 May 2002; JP 2002-158727 31 May 2002
DT Patent
LA Japanese
OS WPI: 2004-286376 [27]
AN 2004-13881 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A human chimeric antibody (IA) or human complementarity determining region (CDR) transplant antibody (IB), specific for prostrate gland specific membrane antigen, each comprising of a heavy chain consisting of variable heavy chain (VH) and constant heavy chain (CH) and a light chain (L) consisting of variable light chain (VL) and constant light chain (CL) or its fragment, is new.

DETAILED DESCRIPTION - A human chimeric antibody or its fragment (IA) specific for prostrate gland specific membrane antigen comprising a heavy chain consisting of variable heavy chain (VH) and constant heavy chain (CH), having a fully defined sequence of 122 amino acids, given in the specification and a light chain (L) consisting of variable light chain (VL) and constant light chain (CL), having a 107 amino acid sequence, given in the specification or human complementarity determining region (CDR) transplant antibody or its fragment (IB) comprising heavy chain consisting of (VH) and (CH) of a human antibody containing CDR1, CDR2, and CDR3 having a sequences (S1) of Ser-Asp-Tyr-Ala-Trp-Asn, Tyr-Ile-Ser-Phe-Ser-Gly-Ser-Thr-Ser-Tyr-Asn-Pro-Ser-Leu-Lys-Ser, Trp-Asn-Tyr-Tyr-Gly-Ser-Ser-His-Val-Trp-Phe-Ala-Tyr, respectively and a light chain consisting of VL and CL of an antibody containing CDR1, CDR2, and CDR3 having sequences (S2) of Lys-Ala-Ser-Gln-Asp-Ile-Tyr-Ser-Tyr-Leu-Ile, Arg-Ala-Asn-Arg-Leu-Val-Asp, Leu-Gln-Tyr-Asp-Glu-Phe-Ala-Thr, respectively. INDEPENDENT CLAIMS are included for the following: (1) DNA (II) which codes (IA) or (IB); (2) recombinant vector (III) containing (II); (3) transformant (IV) obtained by introducing (III) to a host cell; (4) producing (IA) or (IB), comprising culturing (IV) in a culture medium, accumulating (IA) or (IB) in the culture and collecting (IA) or (IB) from the culture; (5) fusion antibody (V) comprising one or more substance chosen from a medical agent, a high molecular compound, a radioisotope and a protein coupled with (IA) or (IB); (6) DNA (VI) which codes (V); (7) recombinant vector (VII) containing (VI); (8) producing (V), comprising culturing (VIII) in a culture medium, coupling a

substance to (IA) or (IB), producing and accumulating (V) in the culture and collecting (V) from the culture; (9) transformant (VIII) obtained by introducing (VII); (10) therapeutic agent of cancer containing one or more type chosen from (IA) or (IB) and (V) as an active ingredient; and (11) diagnostic agent of cancer containing (IA) or (IB) as an active ingredient.

BIOTECHNOLOGY - Preferred Antibody: In (IA) or (IB), the CH is of IgG1 class and CL belongs to kappa class. (IA) is produced by a transformant KM 2777 having an accession number FERM BP-7969. In (IB) VH consists of a frame work region (FR) of CDR1, CDR2 and CDR3 having (S1) and VL consisting of a FR of CDR1, CDR2 and CDR3 having (S2). In (IA) or (IB) the antibody fragment is chosen from the peptide containing Fab, Fab', F(ab')2, single stranded antibody (scFv), dimerization V range (diabody) disulfide stabilization V range (dsFv) and CDR.
Preferred Transformant: (IV) is KM2777 having accession number FERM BP-7969. (VIII) is KM2812 having an accession number FERM BP-7970.
Preferred Fusion Antibody: In (V) the medical agent couple with (IA) or (IB) is an anticancer agent or an antiinflammatory agent. The protein coupled with (IA) or (IB) is one or more protein chosen from a toxin and cytokine. In (V), the substance is coupled with the heavy chain or light chain of (IA) or (IB), preferably coupled with both heavy and light chains. The protein is preferably human interleukin 2 (hiL-2). (V) contains (IA) or (IB) produced by (IV) and hiL-2. (V) is produced by (VIII). In (V) the anticancer agent is an adriamycin. (IA) or (IB) is coupled with adriamycin. Preferred Method: In (M1), the adriamycin joint linker is combined through a dipeptide and polyethylene glycol, which transform converted the amino group of an adriamycin and the hydroxyl group of the terminal into the carboxy group is combined, the carboxy group of the terminal of this linker is activated-esterized, where (IA) or (IB) is made to with the substance (adriamycin).

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Inhibitor of cancer cell proliferation. Adriamycin (anticancer agent) joint anti-prostrate gland specific membrane antigen (PSMA) chimeric antibody was prepared. Reactivity with a human prostatic-cancer cell strain was studied. The human prostatic-cancer cell strain LNCaP of ADM-KM2777 (ATCCCRL-1740) and the cell growth inhibition activity with respect to PC-3 (ATCCCRL -1435) were measured as follows. Each cell was prepared to 1x10 to the power 5 cell/ml by a RPMI (10) culture medium, dispensed by 50 microl/well on the plate for 96 well cultured. Furthermore, the antibody solution was diluted to various concentration by the RPMI(10) culture medium and was added by 50 microl/well, cultured within 37 degreesC and 5 % Co2 incubator for 96 hours. After the culture, cell growth reagent WST-1 was dispensed by 10 microl well, furthermore, after culture within 37 degreesC and 5 % Co2 incubator for 4 hours, plate reader Emax was used and optical density (OD) at 450 nm was measured. The results showed that the ADM-KM2777 had cell growth inhibition activity.

USE - (IA) or (IB) is useful as a diagnostic agent or a therapeutic agent for cancer (claimed). (I) is useful for measuring the concentration of PSMA in blood.

ADMINISTRATION - (IA) or (IB) is administered by oral, intravenous, intramuscular, subcutaneous, parenteral or rectal route in dosages ranging from 10-20 microg/kg/adult/day.

ADVANTAGE - (IA) or (IB) is effective in assay of PSMA. (IA) or (IB) effectively diagnosis or treats cancer, specifically prostrate cancer.

EXAMPLE - Adriamycin (anticancer agent) joint anti-prostrate gland specific membrane antigen (PSMA) chimeric antibody was prepared. Reactivity with a human prostatic-cancer cell strain was studied. The human prostatic-cancer cell strain LNCaP (ATCCCRL-1740) and the 2x10 to the power 5 cell of PC-3 (ATCCCRL-1435) were suspended by 50 micro-l of the solution which diluted ADM-KM2777 and the anti-PSMA chimeric antibody KM2777 to 10 micro-g/ml in

bovine serum albumin (BSA)-phosphate buffered saline (PBS), respectively and was made to react for 30 minutes at 4 degreesC. After reaction, using PBS, centrifuged 3 times and washed. 50 micro-l of solutions diluted with fluorescein-isothiocyanate (FITC) label human IgG (H+L) 20 times by BSA-PBS after that was added, and was made to react for 30 minutes at 4 degreesC after a suspension. After reaction, using PBS, centrifuged 3 times and washed and suspended after that in 1 ml of PBS, and analyzed using flow cytometer. The results showed that ADM-KM2777 showed a PSMA positive LNCaP cell and specific reaction like the anti-PSMA chimeric antibody KM2777, on the other hand, it did not react with PSMA negativity PC-3 cell. Moreover, reactive strength was also equivalent. ADM-KM2777 had avidity equivalent to KM2777 after coupling of ADM to KM2777. (62 pages)

L2 ANSWER 6 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2004-16632 BIOTECHDS
TI The effect of variable domain orientation and arrangement on the antigen-binding activity of a recombinant human bispecific diabody.;
antibody production against growth factor for use in disease therapy
AU LU D; JIMENEZ X; WITTE L; ZHU ZP
CS ImClone Syst Inc; ImClone Syst Inc
LO Zhu ZP, ImClone Syst Inc, Dept Antibody Technol and Mol, New York, NY 10014 USA
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS; (2004) 318, 2, 507-513 ISSN: 0006-291X
DT Journal
LA English
AN 2004-16632 BIOTECHDS
AB AUTHOR ABSTRACT - In recent years a variety of recombinant methods have been developed for efficient production of bispecific antibodies (BsAb) in various formats. Bispecific diabody (bDAb), a 55-60 kDa molecule comprising two non-covalently associated cross-over single chain Fv (scFv) polypeptides, represents one of the most promising as well the most straightforward approaches to BsAb production. Here we constructed a bDAb, using two human scFv, 11F8 and A12, directed against the epidermal growth factor receptor (EGFR) and the insulin-like growth factor receptor (IGFR), respectively, as the building blocks. A total of 8 scFv and diabody constructs were prepared comprising the same two variable heavy (V-H) and variable light (V-L) chain domains but arranged in different orientations. V-H/V-L orientation, i.e., V-H-linker -V-L or V-L-linker-V-H, showed significant effects oil the expression and antigen-binding activity of scFv and monospecific diabody of both 11F8 and A12. Further, only 2 out of the 4 possible V-H/V-L orientations/arrangements in bDAb construction yielded active products that retain binding activity to both EGFR and IGFR. Both active bDAb preparations retained their original antigen-binding activity after incubation at 37 degreesC in mouse serum for up to 7 days, indicating excellent stability of the constructs. Taken together, Our results underscore the importance of identifying/selecting optimal V-H/V-L orientation/arrangement for efficient production of active bDAb. (C) 2004 Elsevier Inc. All rights reserved.
DERWENT ABSTRACT: The single chain antibody (scFv) and diabodies were secreted from Escherichia coli strain HB2151 containing the expression plasmid grown at 30 deg in a shaker flask, a periplasmic extract of the cells was prepared, and the soluble scFv and diabody were purified from the extract by anti-E tag affinity chromatography using the RPAS Purification Module following a procedure previously described. To examine the purity of the preparations, the purified scFv and diabodies were electrophoresed in an 18% polyacrylamide gel Tween 20(7 pages)

L2 ANSWER 7 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2004-08884 BIOTECHDS

TI Immunosuppressive properties of anti-CD3 single-chain Fv and diabody;
antibody production via cell culture against CD3 for use in therapy
AU LE GALL F; REUSCH U; MOLDENHAUER G; LITTLE M; KIPRIYANOV SM
CS Affimed Therapeut AG; German Canc Res Ctr
LO Kipriyanov SM, Affimed Therapeut AG, Neuenheimer Feld 582, D-69120 Heidelberg, Germany
SO JOURNAL OF IMMUNOLOGICAL METHODS; (2004) 285, 1, 111-127 ISSN: 0022-1759
DT Journal
LA English
AN 2004-08884 BIOTECHDS
AB AUTHOR ABSTRACT - The mouse anti-human CD3 monoclonal antibody OKT3 is a potent immunosuppressive agent used in clinical transplantation. However, OKT3 therapy is associated with unpleasant and often serious side effects which appear to result from cytokine release, complement activation and a human anti-mouse antibody (HAMA) response. To decrease these adverse side effects, we constructed antibody fragments comprising OKT3 variable domains without any constant domains. Single-chain Fv (scFv) monomers, dimers and trimers were generated by changing the linker length between the V-H and V-L domains. The linkers used were the natural extensions of the V-H into the C(H)1 domain. The dimeric molecules (diabodies) demonstrated the best CD3-binding activity. The diabody with the six amino acid linker was produced in bacteria with a tenfold higher yield than other scFvs and possessed CD3-binding affinity approaching that of the parental mAb. In contrast to OKT3 mAb, the anti-CD3 diabody and scFv monomer did not cause any T-cell activation and cytokine release in vitro, while demonstrating CD3·modulation. In mixed lymphocyte cultures, both diabody and scFv, but not the monoclonal antibody OKT3, were able to suppress T-cell activation and secretion of IL-2 and IFN-gamma in a dose-dependent manner. The anti-CD3 diabody may provide a potent immunosuppressive drug with low toxicity and immunogenicity. (C) 2004 Elsevier B.V. All rights reserved.
DERWENT ABSTRACT: For antibody production, Escherichia coli K12 strain RV308 was used for functional expression of antibody fragments. The bacteria transformed with the expression plasmids were grown in shaking flasks and induced essentially as described. To isolate soluble periplasmic proteins, the pelleted bacteria were resuspended in 5% of the initial volume of ice-cold 200 mM Tris-HCl, 20% sucrose, 1 mM EDTA, pH 8.0. After 1 hr incubation on ice with occasional stirring, the spheroplasts were pelleted by centrifugation and the supernatant containing the soluble periplasmic proteins was thoroughly dialyzed against 50 mM Tris-HCl, 1 M NaCl, pH 7.0. The recombinant product was concentrated by ammonium sulfate precipitation. The protein precipitate was collected by centrifugation and dissolved in 10% of the initial volume of 50 mM Tris-HCl, 1 M NaCl, pH 7.0, followed by thorough dialysis against the same buffer. Immobilized metal affinity chromatography (IMAC) was performed at 4 deg using a 5-ml column of Chelating Sepharose charged with Cu 2+ and equilibrated with 50 mM Tris-HCI, 1 M NaCl, pH 7.0. The sample was loaded by passing the sample over the column by gravity flow. The column was then washed with 20 column volumes of start buffer followed by start buffer containing 50 mM imidazole until the absorbance of the effluent was minimal. Absorbed material was eluted with 50 mM Tris-HCI, 1 M NaCl, 300 mM imidazole, pH 7.0, as 1 ml fractions. The eluted fractions containing recombinant protein were identified by reducing 12% SDS-PAGE followed by Coomassie staining. The final purification of scFv was achieved by ion-exchange chromatography on a Mono S HR5/5 column(17 pages)

L2 ANSWER 8 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2
AN 2003:832137 CAPLUS
DN 139:322291

TI Antibody combinations for tumor therapy
IN Kipriyanov, Sergey; Le Gall, Fabrice; Cochlovius, Bjoern; Little, Melvyn
PA Affimed Therapeutics A.-G., Germany
SO Eur. Pat. Appl., 23 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 1354600	A1	20031022	EP 2002-8845	20020419
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	WO 2003088998	A1	20031030	WO 2003-EP3928	20030415
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2003224076	A1	20031103	AU 2003-224076	20030415
	US 2005176934	A1	20050811	US 2003-510881	20030415
PRAI	EP 2002-8845	A	20020419		
	WO 2003-EP3928	W	20030415		

AB Disclosed is a combination of at least 2 antibodies, characterized by the following properties: (a) it comprises at least 2 different multivalent antibodies, each one having at least 2 specificities and being characterized by features: (b) + (d) for at least one of said 2 different multivalent antibodies, and (b) + (c) for at least one other of said 2 different multivalent antibodies, where (b) is an antigen-binding domain specific to a tumor antigen, (c) is an antigen-binding domain specific to an antigen present on human T cells, and (d) is an antigen-binding domain specific to an antigen present on CD3-epsilon neg. human effector cells. Also disclosed are polynucleotides encoding said antibodies as well as vectors comprising said polynucleotides, host cells transformed therewith, and their use in the production of said antibodies. Finally, compns., preferably pharmaceutical and diagnostic compns., are disclosed comprising the above mentioned polynucleotides, antibodies, or vectors. The pharmaceutical compns. are useful for immunotherapy, preferably against B cell malignancies, B cell-mediated autoimmune diseases, and diseases associated with depletion of B cells. In a SCID mouse model of established Burkitt's lymphoma receiving the synergistic combination of CD19XCD16 bispecific diabodies, CD19XCD3 bispecific diabodies, and anti-CD28 monoclonal antibodies, animal survival was significantly improved.
RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 9 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2004-00650 BIOTECHDS
TI New antibody fragments, useful for treatment and diagnosis of tumors, that recognize glycosylated carcino-embryonal antigens; antibody production via cell culture for use in disease diagnosis and therapy
AU GAVILONDO COWLEY J V; AYALA AVILA M; FREYRE ALMEIDA F D L M; ACEVEDO CASTRO B E; BELL GARCIA H; ROQUE NAVARRO L T; GONZALEZ LOPEZ L J; CREMATA ALVAREZ J A; MONTESINO SEGUI R
PA CENT ING GENETICA and BIOTECNOLOGIA
PI WO 2003093315 13 Nov 2003

AI WO 2003-CU5 28 Apr 2003
PRAI CU 2002-86 29 Apr 2002; CU 2002-86 29 Apr 2002
DT Patent
LA Spanish
OS WPI: 2003-854481 [79]
AN 2004-00650 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Antibody fragments (A) of the monomeric, single-chain Fv type, obtained from the RNA of the hybridoma that produces monoclonal antibody CB/ior-CEA.1, specific for human carcino-embryonal antigen (CEA), in soluble form, adsorbed on solid surfaces or present in cells, are new. (A) has affinity constant for glycosylated CEA of 5+/- 0.4 x 10000000000 l/mole.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) similar divalent (diabody) fragment (A2) with affinity constant for glycosylated CEA of 2.8 +/- 0.3 x 1010 l/mole; (2) CEA-specific recombinant or synthetic antibody fragments (B) containing the variable heavy and light chain regions of (A) and (A2), artificially linked as Fab or other scFv fragments, bispecific antibodies or fused to biologically or biochemically active domains; (3) cells or multicellular organisms that express (A), (A2) or (B); and (4) vectors that encode (A), (A2) or (B).

BIOTECHNOLOGY - Preferred Proteins: (A) has a 241 amino acid (aa) sequence (16) and (A2) a 232 aa sequence (17), both reproduced, and these sequences are present in (B). (A), (A2) and (B) may also include: (i) a radioactive or other detectable marker; or (ii) a chemical or biological agent with antitumor activity. Preparation: Total RNA was isolated from CB/ior-CEA.1; converted to cDNA and both the light and heavy chain sequences amplified by PCR, using primers (sequences reproduced) that amplify: (i) for the heavy chain, from the signal peptide to the CH1 region; and (ii) for the light chain, from the signal peptide to the Ckappa region. Amplicons (of 320-350 bp) were purified and blunt-end cloned into pMOS for sequencing. Sequences present in the recombinant plasmids pVH5 and pVL2 were amplified by PCR, and the combined amplicons were subjected to a second round of PCR to assemble the sequence that encoded (A). The sequence for (A2) was prepared by essentially the same method, except that for (A) the linker was GluGlyLysSerSerGlySerGlySerGluSerLysValAsp while for (A2) it was Gly4Ser. All primer sequences are reproduced. The final (A)- or (A2)-expressing amplicon was digested with ApaL1 and NotI then cloned into pJG-1m, cut with the same enzymes, and recombinant plasmids expressed in Escherichia coli XL-1Blue. The recombinant plasmids were sequenced; they include inserts that encode (16) (plasmid pJG1m-25) or (17) (plasmid pJG1m-18). Once isolated, the coding sequences may also be expressed in yeast or insect, mammalian and plant cells, or in transgenic animals or plants.

ACTIVITY - Cytostatic. No biological data given.

MECHANISM OF ACTION - Antibody binding to CEA.

USE - (A), and related diabodies (A2), optionally labeled, are used to identify tumor cells that express CEA. Also (A), (A2) and other antibody structures containing the same variable domains, are used, optionally in conjunction with an active agent, for treatment or in vivo localization (imaging) of CEA-expressing tumors, e.g. of colon, lung or breast.

ADVANTAGE - (A) are related fragments retain high affinity for CEA and do not cross-react with normal human tissues or cells (except normal colonic mucosa, where CEA is occasionally present). Since they are smaller, by a factor of 2.5-5 times, than the complete antibody, they have better tissue penetration and are less immunogenic in humans.

EXAMPLE - The carcino-embryonal antigen-expressing tumor cell line B16-CEA was implanted into mice and after 7 days the animals were injected with a new 125-iodine labeled scFv antibody, based on monoclonal antibody CB/ior-CEA.1. The ratios of radioactivity in tumor:radioactivity in blood were 43.60 and 53.50 after

24 and 48 hours, respectively; compare 1.79 and 2.62 for a known scFv (Biotechniques, 13 (1992) 790) derived from the same parent antibody. (49 pages)

L2 ANSWER 10 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2003-19995 BIOTECHDS
TI New multivalent, monospecific binding protein comprising two or more binding sites having affinity for the same single target antigen, where each binding site is associated with scFv fragments, useful for diagnosing or treating tumor;
recombinant vector-mediated gene transfer and expression in host cell for use in diagnosis and gene therapy
AU ROSSI E; CHANG C K; GOLDENBERG D M
PA ROSSI E
PI WO 2003033654 24 Apr 2003
AI WO 2002-US32718 15 Oct 2002
PRAI US 2002-404919 22 Aug 2002; US 2001-328835 15 Oct 2001
DT Patent
LA English
OS WPI: 2003-513460 [48]
AN 2003-19995 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A multivalent, monospecific binding protein comprising two or more binding sites having affinity for the same single target antigen, where the binding sites are formed by the association of two or more single chain Fv (scFV) fragments, and each scFV fragment comprises at least two variable domains derived from a humanized or human monoclonal antibody, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) an expression vector comprising a nucleotide sequence encoding the monospecific diabody, triabody or tetrabody; (2) a host cell comprising the expression vector; (3) diagnosing the presence of a tumor by administering to a subject suspected of having a tumor a detectable amount of the binding protein, and monitoring the subject to detect any binding of the binding protein to tumor; (4) delivering one or more diagnostic and/or therapeutic agents to a tumor by administering the binding protein to the subject; and (5) a kit for therapeutic and/or diagnostic use, comprising the binding protein, and additional reagents, equipments and instructions for use.

BIOTECHNOLOGY - Preferred Protein: The binding protein comprises a monoclonal antibody that is specific for a tumor-associated antigen. The tumor-associated antigen is associated with a disease state selected from a carcinoma, a melanoma, a sarcoma, a neuroblastoma, a leukemia, a glioma, a lymphoma and a myeloma. The tumor-associated antigen is associated with a type of cancer selected from acute lymphoblastic leukemia, acute myelogenous leukemia, biliary, breast, cervical, chronic lymphocytic leukemia, chronic myelogenous leukemia, colorectal, endometrial, esophageal, gastric, head and neck, Hodgkin's lymphoma, lung, medullary thyroid, non-Hodgkin's lymphoma, ovarian, pancreatic, prostate and urinary bladder. The tumor-associated antigen is selected from A3, A33, BrE3, CD1, CD1a, CD3, CD5, CD15, CD19, CD20, CD21, CD22, CD23, CD30, CD45, CD74, CD79a, CEA, CSAp, EGFR, EGP-1, EGP-2, Ep-CAM, Ba 733, HER2/neu, KC4, KS-1, KS1-4, Le-Y, MAGE, MUC1, MUC2, MUC3, MUC4, PAM-4, PSA, PSMA, RS5, S100, T101, TAG-72, tenascin, Tn antigen, Thomson-Friedenreich antigens, tumor necrosis antigens, vascular endothelial growth factor (VEGF), 17-1A, an angiogenesis marker, a cytokine, an immunomodulator, an oncogene marker and an oncogene product. The tumor-associated antigen is carcinoembryonic antigen (CEA). The humanized monoclonal antibody is hMN-14. The binding protein further comprises at least one agent selected from a diagnostic agent, a therapeutic agent and their combinations. The diagnostic agent is selected from a conjugate, a radionuclide, a metal, a contrast agent, a tracking agent, a detection agent, and their combinations. The radionuclide is selected from 11C, 13N, 15O, 18F, 32P, 52Mn, 55Co, 62Cu,

64Cu, 67Ga, 68Ga, 72As, 76Br, 82mRb, 83Sr, 89Zr, 90Y, 94mTc, 94Tc, 99mTc, 110In, 111In, 120I, 123I, 124I, 125I, 131I, Gd, 177Lu, 186Re, 188Re, a gamma-emitter, a beta-emitter, a positron emitter, or their combinations. The metal is selected from gadolinium, iron, chromium, copper, cobalt, nickel, dysprosium, rhenium, europium, terbium, holmium, neodymium, and their combinations. The contrast agent is a MRI contrast agent, a CT contrast agent, or an ultrasound contrast agent. The contrast agent is selected from gadolinium ions, lanthanum ions, manganese ions, iron, chromium, copper, cobalt, nickel, dysprosium, rhenium, europium, terbium, holmium, neodymium, another comparable contrast agent, and their combinations. The tracking agent is selected from iodine compounds, barium compounds, gallium compounds, thallium compounds, barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iodipamide, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosemetic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, thallous chloride, and their combinations. The detection agent is selected from an enzyme, a fluorescent compound, a chemiluminescent compound, a bioluminescent compound, a radioisotope, and their combinations. The therapeutic agent is selected from a radionuclide, a chemotherapeutic drug, a cytokine, a hormone, a growth factor, a toxin, an immunomodulator, and their combination. The chemotherapeutic drug is selected from vinca alkaloids, anthracyclines, epidophyllotoxins, taxanes, antimetabolites, alkylating agents, antibiotics, Cox-2 inhibitors, antimitotics, antiangiogenic agents, apoptotoic agents, doxorubicin, methotrexate, taxol, CPT-11, camptothecans, nitrogen mustards, alkyl sulfonates, nitrosoureas, triazenes, folk acid analogs, pyrimidine analogs, purine analogs, platinum coordination complexes, hormones, and their combinations. The toxin is selected from ricin, abrin, ribonuclease, DNase I, Staphylococcal enterotoxin A, pokeweed antiviral protein, gelonin, diphtherin toxin, Pseudomonas exotoxin or endotoxin, and their combinations. The immunomodulator is selected from cytokines, stem cell growth factors, lymphotoxins, hematopoietic factors, colony stimulating factors, interferons, stem cell growth factors, erythropoietin, thrombopoietin, and their combinations. The humanized monoclonal antibody is hMN-14. Each scFv comprises the VH and VK regions of hMN-14. Each scFv further comprises an amino acid linker connecting the VH and VK regions of hMN-14. This is a monospecific diabody, where each scFv comprises a sequence of 261 amino acids; a monospecific triabody, where each scFv comprises a sequence of 256 amino acids; or a monospecific tetrabody, where each comprises a sequence of 257 amino acids. Preferred Method:

ACTIVITY - Cytostatic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The binding proteins are useful for diagnosing and treating tumors, e.g. carcinoma, a melanoma, a sarcoma, a neuroblastoma, a leukemia, a glioma, a lymphoma and a myeloma; or a cancer selected from acute lymphoblastic leukemia, acute myelogenous leukemia, biliary, breast, cervical, chronic lymphocytic leukemia, chronic myelogenous leukemia, colorectal, endometrial, esophageal, gastric, head and neck, Hodgkin's lymphoma, lung, medullary thyroid, non-Hodgkin's lymphoma, ovarian, pancreatic, prostate and urinary bladder. When treating a tumor by administering to the subject the binding protein, and/or a therapeutic agent , the therapeutic agent is a chemotherapeutic drug, a toxin, external radiation, brachytherapy radiation agent, a radiolabeled protein, an anticancer drug, or an anticancer antibody (all claimed).

ADMINISTRATION - Administration is intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, or intrathecal. No dosage is given.

EXAMPLE - No relevant example given. (62 pages)

L2 ANSWER 11 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

AN 2003-15276 BIOTECHDS
TI New immunoglobulin molecule, useful in therapeutic or diagnostic assays comprising ELISA, phage display, tumor imaging or protein chips assay or in screening assays for detecting molecules that bind to the immunoglobulin molecule;
recombinant antibody production and transgenic plant generation conferring disease-resistance
AU ZHANG M Y; SCHILLBERG S; ZIMMERMANN S; DI FIORE S; EMANS N; FISCHER R
PA FRAUNHOFER INST MOLEKULARBIOLOGIE and ANGE
PI WO 2003025124 27 Mar 2003
AI WO 2002-US29003 13 Sep 2002
PRAI US 2001-318904 14 Sep 2001; US 2001-318904 14 Sep 2001
DT Patent
LA English
OS WPI: 2003-371805 [35]
AN 2003-15276 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Immunoglobulin molecule comprising: (a) one or more heavy chain framework regions comprising HFR1, HFR2, HFR3 or HFR4 and one or more light chain framework regions comprising LFR1, LFR2, LFR3 or LFR4; and (b) complementarity determining regions (CDRs) comprising CDR-H1, CDR-H2, CDR-H3 and/or CDRL1, CDR-L2 or CDR-L3, is new.

DETAILED DESCRIPTION - The immunoglobulin molecule has the structure:(i) HFR1-CDR-H1-HFR2-CDR-H2-HFR3-CDR-H3-HFR4; or (ii) LFR1-CDR-L1-LFR2-CDR-L2-LFR3-CDR-L3-LFR4. The length of, and positions of the amino acid residues in, the CDRs and the framework regions are in accordance with the Kabat numbering system. HFR1 is a first framework region in (B) comprising a sequence of about 30 amino acid residues. HFR2 is a second framework region in (B) comprising a sequence of about 14 amino acid residues. HFR3 is a third framework region in (B) comprising a sequence of about 29-32 amino acid residues. HFR4 is a framework region in (B) comprising a sequence of 7-9 amino acid residues, where the first amino acid is tryptophan (Trp). CDR-H1 is a first complementary determining region. CDR-H2 is a second complementary determining region. CDR-H3 is a third complementary determining region. LFR1 is a first framework region comprising a sequence of about 22-23 amino acid residues. LFR2 is a second framework region comprising a sequence of about 13-16 amino acid residues, where a Pro or Leu must be at position 10 if the sequence is 15 amino acid residues long or position 11 if the sequence is 16 amino acid residues long. LFR3 is a third framework region comprising a sequence of about 32 amino acid residues. LFR4 is a fourth framework region comprising a sequence of about 12-13 amino acid residues, where the first amino acid residue is Phe. CDR-L1 is a first complementary determining region. CDR-L2 is a second complementary determining region. CDR-L3 is a third complementary determining region. INDEPENDENT CLAIMS are also included for: (1) a composition comprising the immunoglobulin molecule; (2) a population of isolated immunoglobulin molecules; (3) an isolated nucleic acid molecule encoding the immunoglobulin molecule; (4) a recombinant library comprising one or more the nucleic acid molecule; (5) a vector comprising the nucleic acid molecule in operable linkage with a promoter; (6) a host cell comprising the nucleic acid molecule; (7) generating a recombinant library of nucleic acid molecules encoding the immunoglobulin molecules having identical framework regions and accumulating to high levels in a host cell; (8) producing a plant resistant to a pathogen; (9) preparing the recombinant library expressing immunoglobulin molecules or their domains; (10) identifying an immunoglobulin molecule of the recombinant library that binds to a predetermined antigen; (11) preparing a transgenic plant; (12) a transgenic plant or its seed; and (13) producing an immunoglobulin molecule having a chimeric variable domain.

BIOTECHNOLOGY - Preferred Immunoglobulin: The immunoglobulin molecule comprises: (i) HRF1, HFR2, HFR3 or HFR4 comprising 30-, 14-, 32- or 9-amino acid sequence, respectively; and (ii) LFR1, LFR2, LFR3 or LFR4 comprising 23-, 16-, 32- or 13-amino acid sequence, respectively. The

immunoglobulin molecule further comprises: (i) a cellular targeting signal and/or a tag; and (ii) a linker that joins (A) to (B). The CDRs are of an avian, piscean or mammalian antibody. It is a VL, Vh, scFV, diabody, triabody or tetrabody. The mammalian antibody is a camelid, murine or human antibody. The cellular targeting signal comprises apoplastic, endoplasmic reticulum, vacuole, protein body or chloroplast targeting peptide. Preferred Composition: The composition is a plant composition. Preferred Population: The population of isolated immunoglobulin molecules is produced by: (i) expressing nucleic acid molecules encoding the immunoglobulin molecules in a host cell to produce a population of immunoglobulin molecules; and (ii) isolating the expressed population of immunoglobulin molecules. Preferred Nucleic Acid: The nucleic acid comprises a sequence having 22 (each of the 2 sequences), 45, 34, 37 or 40 bp. The nucleic acid molecules comprise randomized CDR-encoding sequences. Preferred Promoter: The promoter comprises tissue specific, inducible, constitutive, developmentally regulated or temporarily regulated promoter. Preferred Host Cell: The host cell comprises bacterial, yeast, algae, insect, mammalian or plant cell. It comprises Escherichia coli, CHO or COS cell. It is a monocotyledonous or dicotyledonous plant cell. The monocotyledonous plant cell comprises amaranth, barley, maize, oat, rice, sorghum or wheat. The dicotyledonous plant cell comprises tobacco, tomato, ornamentals, potato, sugarcane, soybean, cotton, canola, alfalfa or sunflower. Preferred Method: Generating a recombinant library of nucleic acid molecules encoding the immunoglobulin molecules having identical framework regions and accumulating to high levels in a host cell comprises: (1) introducing a population of nucleic acid molecules encoding the immunoglobulin molecules comprising avian framework regions into the host cells to generate transformed host cells; (2) assaying the transformed host cells for expression of the nucleic acid molecules; (3) identifying transformed host cells producing levels of immunoglobulin molecules that are at least 0.15% of total cellular protein; (4) isolating the immunoglobulin-encoding nucleic acid molecules from the transformed host cells identified in (3); (5) determining the amino acid sequence of framework regions of the immunoglobulin molecules encoded by the nucleic acid molecules of (4); (6) identifying which amino acid residue positions in the framework regions of (5) are conserved among the immunoglobulin molecules; (7) preparing a consensus sequence for the framework regions of (4) having the conserved amino acid residues identified in (6); and (8) preparing one or more nucleic acid molecules encoding immunoglobulin molecules having the framework regions of (7) and CDRs. Identifying the nucleic acid molecules in (6) that encode an immunoglobulin that binds to a preselected antigen comprises: (1) expressing the nucleic acid molecules to produce an immunoglobulin; (2) assaying the binding of the immunoglobulin to the preselected antigen; and (3) identifying the nucleic acid molecule that encodes the immunoglobulin that binds to the preselected antigen. Producing a plant resistant to a pathogen comprises: (1) transforming a plant cell with the nucleic acid molecule encoding the immunoglobulin molecule specific for the pathogen; (2) regenerating a plant from the transformed cells; and (3) growing the regenerated plant under conditions that promote expression of the nucleic acid molecule; where expression of the nucleic acid molecule confers resistance to the pathogen. The pathogen is virus, bacteria, mycoplasma, fungus, nematode or insect. Preparing the recombinant library expressing immunoglobulin molecules or their domains comprises: (1) preparing one or more nucleic acid molecules encoding the immunoglobulin molecules or their domains; and (2) expressing the nucleic acid molecules in the host cell to produce a recombinant library expressing the immunoglobulin molecules or their domains. Identifying an immunoglobulin molecule of the recombinant library that binds to a predetermined antigen comprises: (1) contacting the immunoglobulin molecules with the

predetermined antigen; and (2) assaying for binding. The method further comprises identifying the nucleic acid molecule that encodes the immunoglobulin or its domain. Preparing a transgenic plant comprises: (1) introducing the nucleic acid molecule into the plant cell to generate a transformed plant cell; (2) regenerating a transgenic plant from the transformed plant cell; and (3) growing the transgenic plant for production of the immunoglobulin molecule from the nucleic acid molecule. Producing an immunoglobulin molecule having a chimeric variable domain comprises: (1) determining amino acid sequence of an avian immunoglobulin molecule; (2) comparing the amino acid sequences of the variable domains of the avian immunoglobulin and the preselected immunoglobulin to identify differences in amino acid residues at corresponding positions in the avian and preselected antibody framework regions and CDRs that are necessary for maintaining conformation of the CDRs; (3) preparing the nucleic acid molecule encoding the immunoglobulin molecule; and (4) expressing the nucleic acid molecule of (3) to produce an immunoglobulin molecule having a chimeric variable domain. The avian immunoglobulin molecule accumulates in a host cell of at least 0.15% total soluble protein. It comprises a sequence having 253-amino acid sequence.

USE - The immunoglobulin is useful in therapeutic or diagnostic assays comprising ELISA, phage display, tumor imaging or protein chips assay. Further, the immunoglobulin is useful in screening assays for detecting molecules that bind to the immunoglobulin molecule (claimed). (198 pages)

L2 ANSWER 12 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2002:804843 CAPLUS
DN 139:51642
TI Recombinant adenoviruses for in vivo expression of antibody fragments
AU Kontermann, Roland E.; Korn, Tina; Jerome, Valerie
CS Vectron Therapeutics AG, Marburg, Germany
SO Methods in Molecular Biology (Totowa, NJ, United States) (2003), 207(Recombinant Antibodies for Cancer Therapy), 421-433
CODEN: MMBIED; ISSN: 1064-3745
PB Humana Press Inc.
DT Journal
LA English
AB The generation of recombinant adenoviruses for the expression of recombinant antibodies, exemplified for bispecific single-chain diabodies (scDb) is described. The first step involves the cloning of antibody as a bivalent diabody introducing a 5 residues linker as well as appropriate cloning ites at the C-terminal region of the VH domain and the N-terminal region of the VL domain. The VH and VL domains of the second antibody are amplified sep. introducing sequences encoding the middle linker M. The resulting scDb contained in a bacterial expression vector is then subcloned into a mammalian expression vector. This expression cassette is subsequently cloned into the pShuttle vector and introduced by homologous recombination into pAdEasy-1. Recombinant adenoviruses are finally purified from the supernatant of transfected HEK293 producer cells.
RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 13 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2002:185179 CAPLUS
DN 136:215405
TI A single-chain antibody to human endoglin for use in the prevention of tumor vascularization
IN Kontermann, Roland; Miller, Daniel; Mueller, Rolf
PA Vectron Therapeutics AG, Germany
SO PCT Int. Appl., 37 pp.
CODEN: PIXXD2

DT Patent
LA German
FAN.CNT 1

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PI	WO 2002020614	A2	20020314	WO 2001-EP10197	20010904
	WO 2002020614	A3	20020801		
	W: CA, JP, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
	DE 10043481	A1	20020411	DE 2000-10043481	20000904
	CA 2421202	AA	20030304	CA 2001-2421202	20010904
	EP 1315760	A2	20030604	EP 2001-980336	20010904
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY, TR				
	JP 2004508035	T2	20040318	JP 2002-525233	20010904
	US 2004053329	A1	20040318	US 2003-363349	20030801
PRAI	DE 2000-10043481	A	20000904		
	WO 2001-EP10197	W	20010904		

AB A single-chain antibody that specifically binds to the extracellular domain of the human endoglin (CD105 antigen) is described for use in the prevention of angiogenesis of tumors. The antibody was identified by screening a phage display library. A fusion protein of the antibody with an antibody to the knob protein of adenovirus 5 is prepared for use in the targeting of adenoviral gene therapy vectors to endothelial cells . Other uses for the antibody, including the use of fusion proteins with anti-CD3 antibodies to induce lysis of endothelial cells.

L2 ANSWER 14 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2003-01892 BIOTECHDS
TI Isolated nucleic acid molecule from a bacterial daptomycin biosynthetic gene cluster encoding a thioesterase or thioesterase domain, useful for generating novel linear and cyclic peptides, and products in a cell;
recombinant enzyme protein production via plasmid expression in host cell use in gene therapy and pharmaceutical and agrochemical product
AU MIAO V P W; BRIAN P; BALTZ R H; SILVA C J
PA MIAO V P W; BRIAN P; BALTZ R H; SILVA C J
PI WO 2002059322 1 Aug 2002
AI WO 2001-US32354 17 Oct 2001
PRAI US 2001-310385 6 Aug 2001; US 2000-240879 17 Oct 2000
DT Patent
LA English
OS WPI: 2002-599794 [64]
AN 2003-01892 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated nucleic acid molecule (I) comprising a sequence that encodes a thioesterase or thioesterase domain, where a gene encoding the thioesterase or thioesterase domain is derived from a bacterial daptomycin biosynthetic gene cluster, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule comprising a sequence that encodes a thioesterase or thioesterase domain; (2) an isolated nucleic acid molecule comprising a part of a sequence that encodes a thioesterase, where the part is at least 13 nucleotides, and where the nucleic acid sequence is derived from a gene from a bacterial daptomycin biosynthetic gene cluster; (3) an isolated nucleic acid molecule comprising a sequence encoding a daptomycin non-ribosomal peptide synthetase (NRPS) or its subunit from Streptomyces; (4) a vector comprising any of the nucleic acid molecules mentioned above; (5) a host cell comprising any of the nucleic acid molecules mentioned above; (6) a host cell comprising the vector of (4); (7) producing a polypeptide selected from a thioesterase, a daptomycin NRPS

or its subunit, comprising culturing the host cell of (5) or (6) under conditions in which the polypeptide is produced, and optionally isolating the polypeptide; (8) an isolated nucleic acid molecule comprising an expression control sequence derived from a gene encoding a thioesterase or daptomycin NRPS derived from a bacterial daptomycin biosynthetic gene cluster, where the molecule is not pRHB153, pRHB157, pRHB159, pRHB160, pRHB166, pRHB168, pRHB172, pRHB599, pRHB602, pRHB603, pRHB680, pRHB613 or pRHB614; (9) a vector comprising the nucleic acid molecule of (8); (10) an isolated polypeptide comprising an amino acid sequence that encodes a thioesterase or its fragment, a daptomycin NRPS or its subunit, module or domain, where they are all derived from a bacterial daptomycin biosynthetic gene cluster; (11) an isolated polypeptide that is encoded by the nucleic acid molecule of (3); (12) an antibody that selectively binds to the polypeptide of (10) or (11); (13) preparing an antibody of (12) comprising immunizing a non-human animal with the polypeptide, and isolating the antibody; (14) determining if a sample contains a nucleic acid molecule encoding a thioesterase, a daptomycin NRPS or its subunit; (15) amplifying a second nucleic acid molecule encoding a thioesterase or its portion from a sample; (16) producing daptomycin comprising introducing a nucleic acid molecule into a host cell; (17) increasing the production of daptomycin by a cell; (18) providing a modified daptomycin comprising culturing a host cell comprising a daptomycin biosynthetic gene cluster with a modified or replaced module; (19) producing a hybrid NRPS or polyketide synthetase (PKS); (20) determining whether a lipopeptide is an antibiotic; (21) identifying a thioesterase; (22) a method to cyclize peptides; (23) a polypeptide comprising any of 42 fully defined sequences of 60-857 amino acids, given in the specification, and encoded by the nucleic acid molecule of (24); (24) a nucleic acid molecule comprising any of 42 nucleotide sequences from 90541 or 12961 bp, fully defined in the specification, and encodes the polypeptide of (23), where the molecule is not pRHB153, pRHB157, pRHB159, pRHB160, pRHB166, pRHB168, pRHB172, pRHB599, pRHB602, pRHB603, pRHB680, pRHB613 or pRHB614; and (25) an antibody that binds to the polypeptide of (23).

WIDER DISCLOSURE - Also disclosed is a computer readable means for storing the nucleic acid and amino acid sequences given above.

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) further comprises the bacterial daptomycin gene cluster to be preferably derived from Streptomyces or S. roseosporus. The molecule is an allelic variant or the thioesterase-encoding domain of nucleotides 78059-85198 or 85537-86352 of 90541 bp, fully defined in the specification. The nucleic acid sequence further comprises a sequence (S1), or where the sequence encodes an amino acid sequence Gly-Trp-Ser-Phe-Gly or Gly-Thr-Ser-Leu-Gly. The nucleic acid molecule of (1) further comprises a nucleic acid sequence selected from: (a) a nucleic acid sequence of dptD or dptH; (b) one encoding a sequence of 2379 or 278 amino acids, fully defined in the specification; (c) a nucleotide sequence from 78059-85198 or 85537-86352 of a fully defined sequence of 90541 bp, given in the specification; (d) a sequence encoding a thioesterase domain of DptD, where the sequence comprises at least a portion of a nucleic acid molecule from dptD, nucleotides 78059-85198 of a sequence of 90541 bp, or a sequence of 2379 bp, all fully defined in the specification; (e) a sequence encoding the amino acid sequence Gly-Trp-Ser-Phe-Gly or Gly-Thr-Ser-Leu-Gly; (f) a sequence comprising any of nucleotides 78488-78511, 79898-79930, 80453-80488, 80558-80581, 80654-80677, 81050-81064, 81623-81646, 83117-83149, 83669-83704, 83774-83797, 83870-83893, 84257-84271, 80033-80320 or 83255-83542 from a sequence of 90541 bp, fully defined in the specification; (g) one encoding an amino acid sequence from amino acids 144-151, 614-624, 799-810, 934-841, 866-873, 998-1002, 1189-1196, 1687-1697, 1871-1882, 1906-1913, 1938-1945, 2067-2071, 659-754 or 1733-1828 of a sequence of 2379 amino acids, fully defined in the specification; (h) a nucleic acid sequence from an S. roseosporus nucleic acid sequence from bacterial artificial chromosome (BAC) clone B12:03A05;

(i) a sequence encoding an amino acid sequence (S2) or (S3); (j) a sequence comprising at least 50 % sequence identity to the nucleic acid sequence of (a)-(h); and (k) a nucleic acid sequence, where a nucleic acid molecule comprising the sequence selectively hybridizes to the complementary strand of a nucleic acid molecule with the nucleic acid sequence of any one of (a)-(h). The homologous molecule of the nucleic acid molecule of (1) or (3) exhibits at least 60%, 70%, 80%, 90% or 95% sequence identity to any of the sequences of (a)-(h). The nucleic acid molecule of (2) further comprises: (a) a nucleic acid sequence encoding DptD or DptH; (b) one encoding a sequence of 2379 or 278 amino acids, fully defined in the specification; (c) a nucleotide sequence from 78059-85198 or 85537-86352 of a fully defined sequence of 90541 bp, given in the specification; (d) a nucleic acid sequence from an S. roseosporus nucleic acid sequence from BAC clone B12:03A05; (e) a nucleic acid sequence further comprises (S1); (f) a sequence comprising any of nucleotides 78488-78511, 79898-79930, 80453-80488, 80558-80581, 80654-80677, 81050-81064, 81623-81646, 83117-83149, 83669-83704, 83774-83797, 83870-83893, 84257-84271, 80033-80320 or 83255-83542 from a sequence of 90541 bp, fully defined in the specification; (g) one encoding an amino acid sequence from amino acids 144-151, 614-624, 799-810, 934-841, 866-873, 998-1002, 1189-1196, 1687-1697, 1871-1882, 1906-1913, 1938-1945, 2067-2071, 659-754 or 1733-1828 of a sequence of 2379 amino acids, fully defined in the specification; (h) a sequence encoding an amino acid sequence (S2) or (S3); (i) a sequence comprising at least 70% sequence identity to any of the sequences of (a)-(e); (j) a sequence that selectively hybridizes to the complement of a nucleic acid sequence of (a)-(e). The part comprises at least 14, 17, 20 or 25 nucleotides of the nucleic acid sequence, and encodes an amino acid sequence comprising the amino acid sequence Gly-Trp-Ser-Phe-Gly or Gly-Thr-Ser-Leu-Gly, or encodes a polypeptide with thioesterase activity. The molecule alternatively comprises an oligonucleotide from 14-60 nucleotides in length. The daptomycin NRPS or subunit in the nucleic acid molecule of (3) is preferably from S. roseosporus. The nucleic acid molecule of (3) is not pRHB153, pRHB157, pRHB159, pRHB160, pRHB166, pRHB168, pRHB172, pRHB599, pRHB602, pRHB603, pRHB680, pRHB613 or pRHB614, where the reference molecule and/or the isolated nucleic acid molecule that has at least 50% sequence identity and/or an allelic variant and/or has at least 14 nucleotides that encodes at least one domain or module from a daptomycin NRPS, comprises a sequence selected from: (a) a sequence selected from dptA, dptB, dptC or dptD; (b) a sequence encoding the amino acid sequence of a polypeptide from DptA, DptB, DptC or DptD, or any of fully defined sequences of 5830, 4105, 3233 or 2379 amino acids, given in the specification; (c) any of the nucleotides from 39555-56047, 56044-68361, 68358-78062 or 78059-85198 from a fully defined nucleic acid sequence of 90541 bp, given in the specification; or (d) a nucleic acid sequence from an S. roseosporus nucleic acid from BAC clone B12:03A05. The nucleic acid molecule of (8) further comprises the bacterial daptomycin gene cluster to be preferably derived from Streptomyces or S. roseosporus. The expression control sequence is derived from the daptomycin NRPS or DptH. The molecule comprises all or part of nucleotides 36018-36407 or 85537-86352 from 90541 bp, fully defined in the specification. The part is at least 30, 50, 100 or 200 nucleotides in length. Preferred Polypeptide: The polypeptide of (10) further comprises: (a) an amino acid sequence from a thioesterase domain of DptD or of a sequence of 2379 amino acids, fully defined in the specification; (b) a sequence of DptH or of 278 amino acids, fully defined in the specification; (c) a sequence encoded by a thioesterase-encoding region of the nucleotides from 78059-85198 or 85537-86352 of 90541 bp, fully defined in the specification; (d) a sequence (S1); (e) a sequence comprising any of nucleotides 78488-78511, 79898-79930, 80453-80488, 80558-80581, 80654-80677, 81050-81064, 81623-81646, 83117-83149, 83669-83704, 83774-83797, 83870-83893, 84257-84271, 80033-80320 or 83255-83542 from a sequence of 90541 bp, fully defined in the specification; (f) a sequence encoding an amino acid

sequence from amino acids 144-151, 614-624, 799-810, 934-841, 866-873, 998-1002, 1189-1196, 1687-1697, 1871-1882, 1906-1913, 1938-1945, 2067-2071, 659-754 or 1733-1828 of a sequence of 2379 amino acids, fully defined in the specification;(g) a nucleic acid sequence from an S. roseosporus nucleic acid sequence from BAC clone B12:03A05; (h) a sequence encoding an amino acid sequence (S2) or (S3); (i) a sequence comprising at least 50% sequence identity to the nucleic acid sequence of (a)-(g); and (j) a nucleic acid sequence, where a nucleic acid molecule comprising the sequence selectively hybridizes to the complementary strand of a nucleic acid molecule with the nucleic acid sequence of any one of (a)-(g). The polypeptide further comprises the bacterial daptomycin gene cluster to be preferably derived from Streptomyces or S. roseosporus. The nucleic acid sequence further comprises a sequence (S1), or where the sequence encodes an amino acid sequence GWSFG or GTSLG. The polypeptide has thioesterase activity, and exhibits at least 60%, 70%, 80%, 90% or 95% sequence identity to any of the sequences of (a)-(g). The polypeptide is a polypeptide fragment, a fusion polypeptide, a polypeptide derivative, a polypeptide analog, a mutein or homologous polypeptide of a naturally-occurring thioesterase derived from a daptomycin synthetic gene cluster. The polypeptide further comprises a fragment with at least 5, 10, 20 or 50 amino acids. The polypeptide is a fusion protein comprising at least 10 or 50 amino acids from the thioesterase. Preferred Vector: The vector of (4) further comprises expression control sequences controlling transcription of the nucleic acid molecule, preferably in a prokaryotic cell. The nucleic acid molecule in the vector of (9) is operatively linked to a second nucleic acid molecule so as to regulate the expression of the second nucleic acid molecule. The second nucleic acid molecule encodes a polypeptide derived from a bacterial daptomycin biosynthetic gene cluster selected from a thioesterase, a daptomycin NRPS or its subunit. The second molecule is a heterologous nucleic acid molecule. Preferred Antibody: The antibody of (12) further comprises an intact immunoglobulin, an antigen-binding portion that is Fab, Fab', F(ab')2, Fv, dAb or a CDR fragment, a single chain antibody, a chimeric antibody, a diabody or a polypeptide with at least a portion of the immunoglobulin sufficient to confer specific antigen binding to the polypeptide. The antibody is a neutralizing or activating antibody. Alternatively, the antibody is a monoclonal or polyclonal antibody. Preferred Method: The method of (14) comprises providing a nucleic acid molecule of (1)-(3), contacting the molecule with the sample under selective hybridization conditions, and determining if the molecule selectively hybridized to a nucleic acid molecule in the sample. The method of (15) further comprises: (a) providing a first nucleic acid molecule, where the first nucleic acid molecule comprises the nucleic acid sequence of (I) and (1), and at least 10 contiguous nucleotides of the nucleic acid sequence; (b) contacting the first nucleic acid molecule with the sample comprising the second nucleic acid molecule under conditions in which the first and second nucleic acid molecules will selectively hybridize to each other; and (c) amplifying the second nucleic acid molecule using polymerase chain reaction (PCR). The method of (16) comprises a daptomycin biosynthetic cluster or its portion to direct the synthesis of daptomycin into a host cell, and culturing the host cell under conditions in which daptomycin is produced. The nucleic acid molecule is preferably derived from Streptomyces or S. roseosporus. The molecule comprises the entire daptomycin biosynthetic gene cluster. The host cell is S. lividans, preferably S. lividans TK64. The method further comprises the step of isolating the daptomycin. The method of (17) comprises providing a host cell that expresses daptomycin, introducing a nucleic acid molecule into a neutral site of a chromosome of the host cell, where introduction of the molecule results in increased production of daptomycin by a cell compared to the cell without the nucleic acid molecule, and culturing the host cell under conditions in which daptomycin is produced. The host cell

is S. roseosporus or S. lividans comprising the daptomycin biosynthetic gene cluster. The nucleic acid molecule is NovA, B, C, dptA, dptB, dptC, dptD, dptD, dptE, dptF, dptG, dptH and fatty acyl-CoA ligase from the daptomycin biosynthetic gene cluster and any combination of two or more nucleic acid molecules. The molecule is a daptomycin resistance gene. The molecule is the entire daptomycin biosynthetic gene cluster or BAC clone B12:03A05. The method further comprises the step of introducing a daptomycin resistance gene into the host cell. The method of (17) further comprises one or more modules specifying incorporation of aspartate to be modified to specify incorporation of asparagine or 3-methyl-glutamate. The module is replaced by a module derived from a non-ribosomal peptide synthetase other than the daptomycin biosynthetic gene cluster. The module specifying incorporation of L-kynurnine is replaced by a module specifying incorporation of L-tryptophan. The method further comprises the step of altering one or more adenylation domains, where the module is inserted directly upstream from a thioesterase module. The thioesterase domain is translocated. The method of (18) comprises: (a) providing a cell comprising a daptomycin biosynthetic gene cluster or its portion to direct the synthesis of daptomycin into a host cell; (b) modifying or replacing, or inserting or deleting, one or more modules of the daptomycin biosynthetic gene cluster or its portion to alter the amino acid that is incorporated into the modified daptomycin, or inserting or translocating a thioesterase domain to the end of an internal module to delete one or more amino acids in the cyclic peptide of the modified daptomycin; and (c) culturing the host cell under conditions in which modified daptomycin is produced. The molecule encoding thioesterase is linked to nucleic acid sequences and not derived from the daptomycin biosynthetic gene cluster and one or more NRPS or PKS. The method is used to produce a novel cyclic peptide or linear peptide. The method of (19) comprises providing a nucleic acid molecule encoding a thioesterase from a daptomycin biosynthetic gene cluster, and linking the nucleic acid molecule encoding a thioesterase to a molecule encoding a natural or synthetic NRPS or PKS. The thioesterase is derived from 78059-85198 or 85537-86352 of 90541 bp, fully defined in the specification. The method of (20) comprises: (a) providing a linear thioester tethered to a cleavable resin; (b) adding a thioester to cyclize the thioester; (c) encapsulating the lipopeptide with a test strain of bacteria; (d) cleaving the cyclic thioester from the resin, and (e) determining if the cyclic thioester has antibiotic activity against the test strain. The resin is a photocleavable resin and the cleaving step is performed using light. The method is used in high throughput screening. The peptide is attached to the resin via a lipid, alkyl or polyether linker.
The method of (21) comprises: (a) providing a linear thioester peptide tethered to a cleavable resin, where the peptide, when cyclized, has antibiotic activity; (b) providing a DNA library in an expression vector that does not lyse a host cell; (c) introducing the DNA library into a host cell that is resistant to the cyclized peptide product; (d) encapsulating the host cell comparing the DNA library and the linear peptide into a matrix to form a macrodroplet; (e) incubating the macrodroplet such that the host cell expresses the polypeptide from the DNA library; (f) placing the macrodroplet on an appropriate target lawn and cleaving the thioester peptide; (g) determining whether the thioester peptide in each macrodroplet has antibiotic activity; and (h) isolating the DNA from the macrodroplet that has antibiotic activity. The method of (22) comprises providing a peptide that contains-and C-terminal amino acid residues that are recognized by a thioesterase derived from a daptomycin biosynthetic gene cluster, and contacting the peptide with the thioesterase under conditions in which cyclization occurs. The peptide is produced by NRPS or PKS, and located within a cell. The thioesterase is encoded by a nucleic acid molecule that has been introduced into the cell. The nucleic acid molecule encoding the thioesterase is operatively linked to a heterologous or to its naturally-occurring promoter.

ACTIVITY - Antibacterial; Fungicide; Virucide; Antiparasitic; Immunomodulator; Antilipemic; Cytostatic.

MECHANISM OF ACTION - Gene therapy.

USE - The compositions and methods of the present invention are useful for generating novel linear and cyclic peptides and improving yield of a product in a cell expressing an NRPS to be used as new compounds or in producing new compounds, such as antibiotics, antifungals, antivirals, antiparasitics, antimitotics, antitumor agents, immunomodulatory agents, anti-cholesterolemic agents, siderophores, agrochemicals and cytostatics.

ADMINISTRATION - The peptides or lipopeptides produced by the compositions and methods can have dosages ranging from 1-25 mg/kg, preferably 1-12 mg/kg body weight, and can be administered by oral, intravenous, intramuscular, subcutaneous, aerosol, topical or parenteral routes.

ADVANTAGE - The methods and compositions provide a first step in producing modified S. roseosporus as well as other host strains which can produce an improved antibiotic having greater potency and in greater quantities, and produce other peptide products having useful biological properties.

EXAMPLE - Mycelium for preparation of megabase DNA was obtained from overnight cultures of S. roseosporus shaken in F10A broth at 30degreesC. Wash cells were embedded in GTG agarose, incubated in lysozyme at 37degreesC of 3 hours, then lysed in 0.1x NLS with 0.2 mg/ml proteinase at 50degreesC overnight to release DNA into the matrix. Gel containing DNA co-migrating with 100-200 kb lambda concatamer size markers was excised and cast in a second gel for an 18 hour run with a 3-5 second linear ramp. DNA estimated at 75-145 kb relative to size markers was electroeluted in TAE. Approximately 2000 clones were archived at 80degreesC in 96-well microtiter plates. (227 pages)

L2 ANSWER 15 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2003-00455 BIOTECHDS
TI Novel immunoglobulin molecule for reducing tumor growth, binds to kinase insert domain-containing receptor with an affinity comparable to human vascular endothelial growth factor, and neutralizes activation of KDR; plasmid-mediated gene transfer for humanized antibody, chimeric antibody and single chain antibody production in COS cell for tumor therapy
AU ZHU Z; WITTE L
PA ZHU Z; WITTE L
PI US 2002064528 30 May 2002
AI US 2001-976787 12 Oct 2001
PRAI US 2001-976787 12 Oct 2001; US 2000-493539 28 Jan 2000
DT Patent
LA English
OS WPI: 2002-589175 [63]
AN 2003-00455 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An immunoglobulin molecule (I) that binds to kinase insert domain-containing receptor (KDR) (a human homolog of mouse fetal liver kinase (FLK)-1 receptor) with an affinity comparable to human vascular endothelial growth factor (VEGF), and that neutralizes activation of KDR, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a nucleic acid molecule (II) encoding: (a) a single chain antibody (Ia), a diabody (Ib), a triabody (Ic) or an antibody (Id) that neutralizes activation of KDR, comprising at least one variable heavy-chain fragment (F1) comprising CDRH1 (comprising a sequence GFNIKDFYMH), CDRH2 (comprising a sequence

WIDPENGDSGYAPKFQG), CDRH3 (comprising a sequence YYGDYEGY) or a sequence (S1) comprising 117 amino acids fully defined in the specification, and at least one variable light-chain fragment (F2) comprising CDRL1 (comprising a sequence SASSSVSYMH), CDRL2 (comprising a

sequence STSNLAS, CDRL3 (comprising a sequence QQRSSYPFT) or a sequence (S2) comprising 108 amino acids fully defined in the specification; or (b) a peptide linker that covalently links F1 and F2; (2) a chimeric or humanized antibody (III) comprising (Id); and (3) making (I) (including (Ia), Ib), (Ic) or (Id)).

BIOTECHNOLOGY - Preparation: (I) (including (Ia), (Ib), (Ic) and (Id)) is obtained by inserting a nucleic acid molecule (II) into a host cell, and expressing the nucleic acid sequence (claimed). Preferred Immunoglobulin: (I) comprises (Ia), (Ib), (Ic) or (Id). F1 (of (Ia)) and F2 (of (Ia)) are covalently linked by at least one peptide linker comprising at least 15 amino acids (a sequence (S3) GGGGSGGGGSGGGGS). F1 (of (Ib)) and F2 (of (Ib)) are covalently linked by a peptide linker comprising at least 5 and not more than 10 amino acids (comprising a sequence (S4) GGGGSGGGGS). (Ib) is monospecific or bispecific, and (Ic) is monospecific, dispecific or trispecific. (Ib) or (Ic) binds to at least one epitope on KDR.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Neutralizes the activation of KDR; inhibitor of angiogenesis (claimed); inhibitor of VEGF-induced mitogenesis. The effect of anti-KDR antibodies on VEGF-stimulated mitogenesis of human endothelial cells was determined with a (3H)-TdR DNA incorporation assay using human umbilical vein endothelial cell (HUVEC). HUVEC (5x103 cells/well) were plated into 96-well tissue culture plates in 200 mul of EBM-2 medium without VEGF, basic fibroblast growth factor (bFGF) or epidermal growth factor (EGF) and incubated at 37degreesC for 72 hour. Various amounts of antibodies were added to duplicate wells and pre-incubated at 37degreesC for 1 hour, after which VEGF165 was added to a final concentration of 16 ng/ml. After 18 hours of incubation, 0.25 muCi of (3H)-TdR was added to each well and incubated for an additional 4 hours. DNA incorporated radioactivity was determined with a scintillation counter. Both c-p1C11 and scFv p1C11 effectively inhibited mitogenesis of HUVEV stimulated VEGF. c-p1C11 was a stronger inhibitor of VEGF-induced mitogenesis of HUVEC than the parent scFv. The antibody concentrations required to inhibit 50% of VEGF-induced mitogenesis of HUVEC were 0.8 nM for c-p1C11 and 6 nM for the scFv, respectively. As expected, scFv p2A6 did not show any inhibitory effect on VEGF-stimulated endothelial cell proliferation.

USE - (I) (including (Ia), (Ib), (Ic) and (Id)) is useful for neutralizing the activation of KDR, reducing tumor growth and inhibiting angiogenesis (claimed).

EXAMPLE - The variable domains of the light (VL) and heavy (VH) chains of p1C11 were cloned from the scFv expression vector by polymerase chain reaction (PCR) using primer 1 (5'CTAGTAGCAACTGCAACTGGAGTACATTCAGACA TCGAGCTC3') and primer 2 (5'TCGATCTAGAAGGATCCACTCACGTTTTATTTCCAG3'), and primer 3 (5'CTAGTAGCAACTGCAACTGGAGTACATTCACAGGTCAAGCTG3') and primer 4 (5'TCGAAGGATCCACTCACCTGAGGAGACGGT3'), respectively. The leader peptide sequence for protein secretion in mammalian cells was then added 5' to VL and VH by PCR using primer 2 and primer 5 (5'GGTCAAAAGCTTATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACT3'), and primers 5 and 4, respectively. Separate vectors for expression of chimeric IgG light chain and heavy chains were constructed. The cloned VL gene was digested with HindIII and BamHI, and ligated into the vector pKN100 containing the human kappa light chain constant region (CL) to create the expression vector for the chimeric p1C11 light chain, c-p1C11-L. The cloned VH gene was digested with HindIII and BamHI, and ligated into the vector pGID105 containing the human IgG1 (gamma) heavy chain constant domain (CH) to create the expression vector for the chimeric p1C11 heavy chain, c-p1C11-H. Both constructs were examined by restriction enzyme digestion and verified by dideoxynucleotide sequencing. Both the VH and the VL domains were precisely fused on their 5' ends to a gene segment encoding a leader peptide sequence as marked. The VH and the VL domains were ligated through HindIII/BamHI sites into expression vector pG1D105 containing a cDNA version of the human gamma1 constant region gene, and pKN 100 containing a cDNA version of the human kappa chain constant

region gene, respectively. In each case, expression was under the control of the HCMVi promoter and terminated by an artificial termination sequence. COS cells were co-transfected with equal amounts of c-p1C11-L and c-p1C11-H plasmids for transient IgG expression. (34 pages)

L2 ANSWER 16 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2002-03837 BIOTECHDS
TI Autocrine costimulation: tumor-specific CD28-mediated costimulation of T cells by in situ production of a bifunctional B7-anti-CEA diabody fusion protein;
expression in 293 cell and Jurkat cell for cancer gene therapy
AU Blanco B; Holliger P; *Alvarez-Vallina L
CS Univ.Madrid; Med.Res.Counc.
LO Department of Immunology, Hospital Universitario Clinica Puerta de Heirro, San Martin de Porres, 4, 28035, Madrid, Spain.
Email: lalvarezv@hpth.insalud.es
SO Cancer Gene Ther.; (2002) 9, 3, 275-81
CODEN: 2815V ISSN: 0929-1903
DT Journal
LA English
AN 2002-03837 BIOTECHDS
AB Expression on a bifunctional fusion protein containing the extracellular portion of B7-1 fused to a five linker diabody specific for human anticarcinoembryonic antigen (CEA) from modified T-lymphocytes was studied. Plasmid pLAV32 expressing the fusion protein directed against colon carcinoma-specific marker CEA, was constructed and expressed in 293 cells and human Jurkat cells.
Different cocultures of transformed Jurkat cells and untransformed Jurkat cells with CEA-negative and CEA-postive cell lines were used. In culture with CEA+ tumor cell, autocrine and paracrine secretion of B7-alpha-CEA provided a potent tumor-specific costimulatory signal to T-lymphocytes in combination with recombinant alpha-CEAxalpha-CD3 bispecific diabody.
B7-alpha-CEA was also shown to enhance survival and tumor-specific activation of T-lymphocytes expressing an anti-CEATRC-based chimeric immune receptor. The above results show that this strategy could be used to increase antitumor activity of immunotherapeutic approaches targeting the T-lymphocyte antigen receptor pathway. (29 ref)

L2 ANSWER 17 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2002-00819 BIOTECHDS
TI Multimerization of a chimeric anti-CD20 single chain Fv-Fc fusion protein is mediated through variable domain exchange;
single chain antibody engineering for potential use in immunotherapy or radioimmunotherapy of lymphoma
AU Wu A M; Tan G J; Sherman M A; Clarke P; Olafsen T; Forman S J; Raubitschek A A
CS Beckman-Res.Inst.; City-of-Hope-Nat.Med.Cent.Duarte
LO Department of Molecular Biology, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010, USA.
SO Protein Eng.; (2001) 14, 12, 1025-33
CODEN: PRENE9 ISSN: 0269-2139
DT Journal
LA English
AN 2002-00819 BIOTECHDS
AB A series of single chain anti-CD20 antibodies was produced in mouse myeloma NS0 cells by fusing single chain Fv (scFv) with human IgG1 hinge and Fc regions, designated scFv-Fc. The initial scFv-Fc construct was assembled using an 18-amino acid linker between the antibody light (VL) and heavy (VH) chain variable regions, with the Cys residue in the upper hinge region (Kabat 233) mutagenized to Ser. Anti-CD20 scFv-Fc retained specific binding to CD20-positive cells and was active in mediating complement-dependent cytolysis.

Variant scFv-Fcs were constructed incorporating 4 different hinges between the scFv and Fc regions or 3 different linkers in the scFv domain. However, like the original construct, these formed multimers. Elimination of a salt bridge between residues Leu38 and His89 in the VL-VH domain interface did not reduce the formation of higher order forms. Structural analysis suggested that the scFv units cross-paired to form a diabody. Thus, domain exchange or cross-pairing appeared to be the basis of the observed multimerization. The single chain antibody was produced for potential use in immunotherapy or radioimmunotherapy of lymphoma. (43 ref)

L2 ANSWER 18 OF 28 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
AN 2001:32662527 BIOTECHNO
TI Construction, expression and characterisation of a single-chain diabody derived from a humanised anti-Lewis Y cancer targeting antibody using a heat-inducible bacterial secretion vector
AU Power B.E.; Caine J.M.; Burns J.E.; Shapira D.R.; Hattarki M.K.; Tahtis K.; Lee F.-T.; Smyth F.E.; Scott A.M.; Kortt A.A.; Hudson P.J.
CS B.E. Power, CSIRO Health Sciences and Nutrition, 343 Royal Parade, Parkville, Vic. 3052, Australia.
E-mail: barbara.power@hsn.csiro.au
SO Cancer Immunology, Immunotherapy, (2001), 50/5 (241-250), 34 reference(s)
CODEN: CIIMDN ISSN: 0340-7004
DT Journal; Article
CY Germany, Federal Republic of
LA English
SL English
AB A single-chain antibody fragment (scFv) of the humanised monoclonal antibody, hu3S193, that reacts specifically with Le.sup.y antigen expressed in numerous human epithelial carcinomas was constructed. A five-residue linker joined the C-terminus of the V.sub.H and the N-terminus of the V.sub.L, which prevented V-domain association into a monomeric scFv and instead directed non-covalent association of two scFvs into a dimer or diabody. The diabody was secreted into the E. coli periplasm using a heat-inducible vector, pPOW3, and recovered as a soluble, correctly processed protein, following osmotic shock or solubilised with 4M urea from the insoluble fraction. The diabody from both fractions was isolated by a rapid batch affinity chromatography procedure, using the FLAG affinity tag to minimise degradation and aggregation. The purified diabody has an M.sub.r of .apprx.54 kDa, was stable and demonstrated similar binding activity as the parent monoclonal antibody, as measured by FACS and BIAcore analyses. The radio-labelled diabody showed a rapid tumour uptake, with fast blood clearance, proving it to be an excellent potential candidate as a tumour-imaging agent.

L2 ANSWER 19 OF 28 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN DUPLICATE
AN 2000:30952149 BIOTECHNO
TI Targeting of bivalent anti-ErbB2 diabody antibody fragments to tumor cells is independent of the intrinsic antibody affinity
AU Nielsen U.B.; Adams G.P.; Weiner L.M.; Marks J.D.
CS J.D. Marks, Dept. Anesthesiol./Pharmaceut. Chem., San Francisco General Hospital, University of California, 1001 Potrero Avenue, San Francisco, CA 94110, United States.
E-mail: Marksj@anesthesia.ucsf.edu
SO Cancer Research, (2000), 60/22 (6434-6440), 29 reference(s)
CODEN: CNREA8 ISSN: 0008-5472
DT Journal; Article
CY United States
LA English
SL English
AB In immunodeficient mice antitumor single-chain Fv (scFv) molecules penetrate tumors rapidly and have rapid serum clearance, leading to

excellent tumor:normal organ ratios. However, the absolute quantity of scFv retained in the tumor is low due to rapid serum clearance and monovalent scFv binding. We previously demonstrated that the presence of an additional binding site prolongs in vitro and in vivo association of scFv-based molecules with tumor cells expressing relevant antigen. The contribution of the intrinsic affinity of each component scFv to the association between a dimeric scFv and its target antigen is largely unknown. Here, we have constructed bivalent diabody molecules from three affinity mutants of the human anti-ErbB2 (HER2/neu) scFv molecule C6.5 by shortening the peptide linker between the heavy (V(H)) and light (V(L)) chains variable domains from 15 to 5 amino acids. The shorter linker prevents intramolecular pairing of V(H) and V(L), resulting in intermolecular pairing and creation of a dimeric M(r) 50,000 molecule with two antigen-binding sites. The scFv used to create the diabodies span a 133-fold range of affinity for the same epitope of ErbB2 [133 nM (C6G98A), 25 nM (C6.5), and 1 nM (C6ML3-9)] and differ by only one to three amino acids. Diabody binding kinetics were determined by surface plasmon resonance on the immobilized ErbB2 extracellular domain. The association rate constants obtained for each diabody molecule were similar to that of the parental (component) scFv. However, the dissociation rate constants obtained for the bivalent diabodies were up to 15-fold slower. The magnitude of the decrease in the bivalent dissociation rate constant was inversely proportional to the monovalent interaction, ranging from only 3-fold for that of the C6ML3-9 diabody to 15-fold for the C6G98A diabody. This resulted in only a 22-fold difference in bivalent affinity, compared with a 133-fold difference in affinity for the respective scFv. Equilibrium-binding constants obtained by surface plasmon resonance correlated well with the equilibrium-binding constants determined in vitro on ErbB2 overexpressing cells. Biodistribution studies were performed in scid mice bearing established SKOV3 tumors. At 24 h, 3-37-fold more diabody was retained in tumor compared with the parental scFv monomers. This likely results from a higher apparent affinity, because of bivalent binding, and a slower serum clearance. Surprisingly, the differences in affinity between diabodies did not result in differences in quantitative tumor retention or tumor to blood ratios. In fact, the diabody constructed from the lowest affinity scFv exhibited the best tumor-targeting properties. We conclude that, above a threshold affinity, other factors regulate quantitative tumor retention. In addition, straightforward dimerization of a low-affinity scFv leads to significantly greater tumor localization than does exhaustive scFv affinity maturation.

L2 ANSWER 20 OF 28 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
AN 2000:30802056 BIOTECHNO
TI Designer genes: Recombinant antibody fragments for biological imaging
AU Wu A.M.; Yazaki P.J.
CS A.M. Wu, Department of Molecular Biology, Beckman Res. Inst. of City of Hope, 1450 East Duarte Road, Duarte, CA 91010, United States. E-mail: awu@coh.org
SO Quarterly Journal of Nuclear Medicine, (2000), 44/3 (268-283), 93 reference(s)
CODEN: QJNMF7 ISSN: 1124-3937
DT Journal; General Review
CY Italy
LA English
SL English
AB Monoclonal antibodies (MAbs), with high specificy and high affinity for their target antigens, can be utilized for delivery of agents such as radionuclides, enzymes, drugs, or toxins in vivo. However, the implementation of radiolabeled antibodies as 'magic bullets' for detection and treatment of diseases such as cancer has required addressing several shortcomings of murine MAbs. These include their

immunogenicity, sub-optimal targeting and pharmacokinetic properties, and practical issues of production and radiolabeling. Genetic engineering provides a powerful approach for redesigning antibodies for use in oncologic applications in vivo. Recombinant fragments have been produced that retain high affinity for target antigens, and display a combination of rapid, high-level tumor targeting with concomitant clearance from normal tissues and the circulation in animal models. An important first step was cloning and engineering of antibody heavy and light chain variable domains into single-chain Fvs (molecular weight, 25-27 kDa), in which the variable regions are joined via a synthetic linker peptide sequence. Although scFvs themselves showed limited tumor uptake in preclinical and clinical studies, they provide a useful building block for intermediate-sized recombinant fragments. Covalently linked dimers or non-covalent dimers of scFvs (also known as diabodies) show improved targeting and clearance properties due to their higher molecular weight (55 kDa) and increased avidity. Further gains can be made by generation of larger recombinant fragments, such as the minibody, an scFv-C(H)3 fusion protein that self-assembles into a bivalent dimer of 80 kDa. A systematic evaluation of scFv, diabody, minibody, and intact antibody (based on comparison of tumor uptakes, tumor:blood activity ratios, and calculation of an Imaging Figure of Merit) can form the basis for selection of combinations of recombinant fragments and radionuclides for imaging applications. Ease of engineering and expression, combined with novel specificities that will arise from advances in genomic and combinatorial approaches to target discovery, will usher in a new era of recombinant antibodies for biological imaging.

L2 ANSWER 21 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN
AN 1999:690871 CAPLUS
DN 131:321540
TI Preparation of single chain, multiple antigen-binding antibodies and their application for assays, diagnosis and therapy
IN Kontermann, Roland; Sedlacek, Hans-harald; Muller, Rolf
PA Hoechst Marion Roussel Deutschland GmbH, Germany
SO Eur. Pat. Appl., 22 pp.
CODEN: EPXXDW
DT Patent
LA German
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 952218	A2	19991027	EP 1999-106176	19990408
	EP 952218	A3	20001018		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	DE 19816141	A1	19991014	DE 1998-19816141	19980409
	DE 19827239	A1	19991223	DE 1998-19827239	19980618
PRAI	DE 1998-19816141	A	19980409		
	DE 1998-19827239	A	19980618		

AB The invention concerns single chain multispecific binding antibodies containing light and heavy chain fragments of Igs with different specificities, VH(A), VL(B), VH(B), VL(A); the light and heavy chains are tethered together by a linker peptide, L; the VH-VL constructs are linked by a peptide, P; alternatively the mol. contains and effector component E, that is linked by a binding fragment B; the mol. can be used for immunoassays, diagnosis and therapy. The single chain diabody mol. has the following scheme: NH2-VH(A)-L-VL(B)-P-VH(B)-L-VL(A)-B-E-COOH. Peptide L contains 5 amino acids; peptide P contains 14-15 amino acids. Peptide B is a protease cleavage sequence, e.g. PSA, cathepsin. Typical specificities of A and B are: target cell, cell membrane, lymphocytes, macrophages, endothelial cells, tumor cells, cytokines, blood coagulation factors, peptide hormones, steroid hormones, histamine, serotonin, etc. Specificity B and/or the

effector component can be directed to an enzyme, fluorescent or radioactive label. The invention also concerns nucleotide sequences coding for the single chain, multiple antigen-binding antibodies (sequences not given), and the 5' start codon of the sequences. Thus a bispecific diabody was constructed to carcinoembryonic antigen (CEA) and E.coli β-galactosidase with the a Myc epitope to 9E10 antibody and a polyhis tag; and expressed in E.coli TG1. The purified protein was 60 kD; 2-300 μg/L protein was fermented. The protein was used to bind to CEA expressing LoVo cells, detection was performed via β-galactosidase reaction with X-Gal substrate; also in an ELISA it reacted with CEA and β-galactosidase.

L2 ANSWER 22 OF 28 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 1999-09905 BIOTECHDS
TI Di-, tri- and tetrameric single chain Fv antibody fragments against human CD19: effect of valency on cell binding;
human CD19-specific single chain antibody, promising for the immunotherapy of B-lymphocyte malignancies such as non-Hodgkin lymphoma
AU le Gall F; Kipriyanov S M; Moldenhauer G; *Little M
CS German-Cancer-Res.Inst.
LO Recombinant Antibody Research Group (D0500), German Cancer Research Center (GKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany. Email: m.little@dkfz-heidelberg.de
SO FEBS Lett.; (1999) 453, 1-2, 164-68
CODEN: FEBLAL ISSN: 0014-5793
DT Journal
LA English
AN 1999-09905 BIOTECHDS
AB Single chain antibody fragments (I) of the mouse monoclonal antibody HD37 specific for human B-lymphocyte antigen CD19 were constructed by joining the variable heavy and light domains with linkers of 18, 10, 1 and 0 residues. The plasmid pHOG21-alpha-CD19 carrying a single chain antibody fragment with an amino acid linker specific for human CD19 was used as a starting material for all of the constructs. Following expression in Escherichia coli K12 strain XL1-Blue, (I)-18 formed mainly monomers and dimers with small amounts of tetramers; (I)-10 formed dimers and some tetramers; (I)-1 only formed tetramers; and (I)-0 formed exclusively trimers. The affinities of the (I)-10 diabody and (I)-I tetrabody were around 1.5- and 2.5-fold higher, respectively, than that of the (I)-0 triabody. At 37 deg, the tetrabody exhibited a significantly prolonged association with cell bound antigen (the half life was 26.6 min) in comparison to both the diabody (13.3 min) and triabody (6.7 min). This improved affinity together with its larger size could be very useful for imaging and for the immunotherapy of B-lymphocyte malignancies. (22 ref)

L2 ANSWER 23 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 4
AN 1999:586528 CAPLUS
DN 132:121276
TI Enzyme recruitment and tumor cell killing in vitro by a secreted bispecific single-chain diabody
AU Brusselbach, S.; Korn, T.; Volkel, T.; Muller, R.; Kontermann, R. E.
CS Institut fur Molekularbiologie und Tumorforschung, Philipps-Universitat Marburg, Marburg, D-35033, Germany
SO Tumor Targeting (1999), 4(2), 115-123
CODEN: TUTAF9; ISSN: 1351-8488
PB Stockton Press
DT Journal
LA English
AB The authors describe the engineering of a bispecific single-chain diabody secreted from mammalian producer cells for recruitment of enzyme to tumor cells. A bispecific diabody (Db-CEAGal) directed against carcinoembryonic antigen

(CEA) and E. coli β-galactosidase (Gal) was converted to a single-chain diabody (scDb-CEAGal) by covalently joining the VHA-VLB and the VHB-VLA fragments with a 15 amino acids flexible linker. Bacterially expressed scDb-CEAGal functionally recruited β-galactosidase to plastic-bound CEA as well as the CEA-expressing colon carcinoma cell line LoVo. Compared to dimeric diabody CEAGal, the single-chain version showed an increased stability in serum at 37°. Active scDb-CEAGal was secreted from stably transfected human cells (HEK 293), specifically recruited β-galactosidase to co-cultivated LoVo cells and mediated tumor cell killing via conversion of the prodrug daunomycin-β-D-galactopyranoside to the toxic drug daunomycin. Single-chain diabodies secreted from mammalian producer cells may be useful for gene-directed antibody therapy by combining antibody-mediated effector recruitment with a gene-therapeutic approach.

RE.CNT 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 24 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 5
AN 1999:634840 CAPLUS
DN 132:21966
TI Bispecific tandem diabody for tumor therapy with improved antigen binding and pharmacokinetics
AU Kipriyanov, Sergey M.; Moldenhauer, Gerhard; Schuhmacher, Jochen; Cochlovius, Bjorn; Von der Lieth, Claus-Wilhelm; Matys, E. Ronald; Little, Melvyn
CS Recombinant Antibody Research Group, German Cancer Res. Cent., Heidelberg, D0500, Germany
SO Journal of Molecular Biology (1999), 293(1), 41-56
CODEN: JMOBAK; ISSN: 0022-2836
PB Academic Press
DT Journal
LA English
AB To increase the valency, stability and therapeutic potential of bispecific antibodies, we designed a novel recombinant mol. that is bispecific and tetravalent. It was constructed by linking four antibody variable domains (VHand VL) with specificities for human CD3 (T cell antigen) or CD19 (B cell marker) into a single chain construct. After expression in Escherichia coli, intramolecularly folded bivalent bispecific antibodies with a mass of 57 kDa (single chain diabodies) and tetravalent bispecific dimers with a mol. mass of 114 kDa (tandem diabodies) could be isolated from the soluble periplasmic exts. The relative amount of tandem diabodies proved to be dependent on the length of the linker in the middle of the chain and bacterial growth conditions. Compared to a previously constructed heterodimeric CD3+CD19 diabody, the tandem diabodies exhibited a higher apparent affinity and slower dissociation from both CD3+and CD19+cells. They were also more effective than diabodies in inducing T cell proliferation in the presence of tumor cells and in inducing the lysis of CD19+ cells in the presence of activated human PBL. Incubated in human serum at 37 °C, the tandem diabody retained 90 % of its antigen binding activity after 24 h and 40 % after one week. In vivo expts. indicated a higher stability and longer blood retention of tandem diabodies compared to single chain Fv fragments and diabodies, properties that are particularly important for potential clin. applications. (c) 1999 Academic Press.

RE.CNT 65 THERE ARE 65 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 25 OF 28 Elsevier BIOBASE COPYRIGHT 2006 Elsevier Science B.V. on STN
AN 1999098745 ESBIOBASE

TI Production and characterization of an anti-(MUC1 mucin) recombinant diabody

AU Denton G.; Brady K.; Lo B.K.C.; Murray A.; Graves C.R.L.; Hughes O.D.M.; Tendler S.J.B.; Laughton C.A.; Price M.R.

CS G. Denton, Cancer Research Laboratories, School of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom.

SO Cancer Immunology Immunotherapy, (1999), 48/1 (29-38), 31 reference(s)
CODEN: CIIMDN ISSN: 0340-7004

DT Journal; Article

CY Germany, Federal Republic of

LA English

SL English

AB A recombinant diabody fragment based on the anti-MUC1 monoclonal antibody, C595 has been produced in a bacterial expression system. Substitution of a 7-amino-acid linker sequence (Gly.sub.6Ser) for the original single-chain (sc)Fv 15-amino-acid linker (Gly.sub.4.sub.-Ser).sub.3, using polymerase-chain-reaction-based strategies, forces variable heavy (V(H)) and light (V(L)) domains to pair with complementary domains on neighbouring scFv molecules, forming a scFv dimer (diabody). This recombinant protein shows similar binding characteristics to the parental C595 monoclonal antibody. The ability to bind to MUC1 mucin on carcinoma cell surfaces will allow its potential as a diagnostic and therapeutic reagent of clinical utility to be investigated.

L2 ANSWER 26 OF 28 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN

AN 1998:28180378 BIOTECHNO

TI Construction and characterization of a bispecific for retargeting T cells to human carcinomas

AU Helfrich W.; Kroesen B.J.; Roovers R.C.; Westers L.; Molema G.; Hoogenboom H.R.; De Leij L.

CS L. De Leij, Department of Clinical Immunology, University Hospital, 1 Hanzeplein, 9713 GZ Groningen, Netherlands.
E-mail: l.f.m.h.de.leij@med.rug.nl

SO International Journal of Cancer, (13 APR 1998), 76/2 (232-239), 23 reference(s)
CODEN: IJCNAW ISSN: 0020-7136

DT Journal; Article

CY United States

LA English

SL English

AB We describe the construction of a recombinant bispecific antibody fragment in the diabody format with specificity for both the well-established human pancarcinoma associated target antigen EGP2 (epithelial glycoprotein 2, also known as the CO17-IA antigen or KSA) and the CD3ε chain of human TCR/CD3 complex. The murine anti-EGP2 (MOC31) single chain variable fragment (scFv) and the humanized anti-CD3 (Uchtlv9) scFv were cast into a diabody format (designated Dia5v9) using a short 5 amino acid Gly-Ser linker between immunoglobulin heavy-chain and light-chain variable domains. Purification of the poly-histidine tagged Dia5v9 was achieved from extracts of protease deficient Escerichia coil by IMAC chromatography. The Dia5v9 diabody showed strong binding to both EGP2 and CD3 in transfected cells. The in vitro efficacy of Dia5v9 in mediating tumor cell lysis by interleukin-2 activated human T cells appeared to be similar to that of the hybrid-hybridoma-derived BsF(ab').sub.2 Bis I (anti-EGP2/anti-CD3) in a standard 4-hr .sup.5.sup.1Cr-release assay. This small and partially humanized recombinant bispecific antibody fragment may be valuable for T-cell-based immunotherapeutical treatment protocols, retargeting activated peripheral blood T lymphocytes to lyse various human carcinomas in vivo.

L2 ANSWER 27 OF 28 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
DUPLICATE
AN 1996:26148310 BIOTECHNO
TI Specific killing of lymphoma cells by cytotoxic T-cells mediated by a bispecific diabody
AU Holliger P.; Brissinck J.; Williams R.L.; Thielemans K.; Winter G.
CS MRC Centre for Protein Engineering, Hills Road,Cambridge CB2 2QH, United Kingdom.
SO Protein Engineering, (1996), 9/3 (299-305)
CODEN: PRENE0 ISSN: 0269-2139
DT Journal; Article
CY United Kingdom
LA English
SL English
AB Antibody fragments produced by bacterial fermentation lack natural effector functions. Bispecific antibody fragments, however, can be endowed with effector functions, for example cell-mediated killing, by binding to and retargeting of cytotoxic cells. Diabodies are a class of engineered antibody fragments with two antigen binding sites, consisting of two associated chains; each chain consists of heavy and light chain variable domains linked by a short polypeptide linker. In contrast to IgG, or other antibody fragments in which the two binding sites can take up a range of orientations and spacings, the diabody structure is more rigid and compact, with the two binding sites separated by 65 Å (less than half the distance in IgG). To establish whether diabodies could also be used in cell-mediated killing, we have explored the use of a bispecific diabody binding to an idiotypic marker on mouse B-cell lymphoma (BCL-1) and to mouse CD3. The bispecific diabody activated naive T-cells and also mediated the specific killing of the lymphoma cells by cytotoxic T-cells. The diabody was less active in T-cell activation but 10-fold more active (w/v) in killing than an analogous bispecific IgG.

L2 ANSWER 28 OF 28 CAPLUS COPYRIGHT 2006 ACS on STN
AN 1994:506521 CAPLUS
DN 121:106521
TI Oligomeric, multispecific derivatives of immunoglobins and their preparation and use
IN Holliger, Kaspar-Philipp; Griffiths, Andrew David; Hoogenboom, Hendricus Renerus J.; Malmqvist, Magnus; Marks, James David; McGuinness, Brian Timothy; Pope, Anthony Richard; Prospero, Terence Derek; Winter, Gregory Paul
PA Cambridge Antibody Technology Ltd., UK; Medical Research Council
SO PCT Int. Appl., 179 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9413804	A1	19940623	WO 1993-GB2492	19931203
	W: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	CA 2150262	AA	19940623	CA 1993-2150262	19931203
	AU 9456548	A1	19940704	AU 1994-56548	19931203
	AU 690528	B2	19980430		
	EP 672142	A1	19950920	EP 1994-902030	19931203
	EP 672142	B1	20010228		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				

	JP 08504100	T2	19960507	JP 1994-513922	19931203
	JP 3720353	B2	20051124		
	AT 199392	E	20010315	AT 1994-902030	19931203
	ES 2156149	T3	20010616	ES 1994-902030	19931203
	CA 2155335	AA	19940818	CA 1994-2155335	19940204
	CA 2155335	C	20010605		
	WO 9418227	A2	19940818	WO 1994-DK54	19940204
	WO 9418227	A3	19941013		
	W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, UZ, VN				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9460380	A1	19940829	AU 1994-60380	19940204
	AU 674568	B2	19970102		
	EP 686162	A1	19951213	EP 1994-906891	19940204
	EP 686162	B1	20030528		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	JP 08506243	T2	19960709	JP 1994-517544	19940204
	JP 3695467	B2	20050914		
	AT 241642	E	20030615	AT 1994-906891	19940204
	ES 2199959	T3	20040301	ES 1994-906891	19940204
	CA 2169620	AA	19950330	CA 1994-2169620	19940916
	WO 9508577	A1	19950330	WO 1994-GB2019	19940916
	W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ				
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	AU 9476214	A1	19950410	AU 1994-76214	19940916
	AU 680685	B2	19970807		
	EP 720624	A1	19960710	EP 1994-926336	19940916
	EP 720624	B1	19981125		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	JP 09503759	T2	19970415	JP 1995-509628	19940916
	AT 173740	E	19981215	AT 1994-926336	19940916
	ES 2126145	T3	19990316	ES 1994-926336	19940916
	CA 2177367	AA	19950608	CA 1994-2177367	19941205
	WO 9515388	A1	19950608	WO 1994-GB2662	19941205
	W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ				
	RW: KE, MW, SD, SZ, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9511170	A1	19950619	AU 1995-11170	19941205
	AU 690171	B2	19980423		
	EP 731842	A1	19960918	EP 1995-902235	19941205
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	NO 9502989	A	19951003	NO 1995-2989	19950728
	NO 316274	B1	20040105		
	FI 9503705	A	19950803	FI 1995-3705	19950803
	FI 113272	B1	20040331		
	US 6589527	B1	20030708	US 1996-621038	19960322
	US 5837242	A	19981117	US 1996-448418	19960514
	US 6492123	B1	20021210	US 1998-146979	19980903
PRAI	GB 1992-25453	A	19921204		
	GB 1993-816	A	19930116		
	EP 1993-303614	A	19930510		
	GB 1993-19969	A	19930922		
	DK 1993-130	A	19930204		
	DK 1993-139	A	19930205		
	WO 1993-GB2492	W	19931203		

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     WO 1994-DK54          W     19940204
     GB 1994-12147         A     19940617
     GB 1994-12166         A     19940617
     WO 1994-GB2019        W     19940916
     WO 1994-GB2662        W     19941205
     US 1996-448418        A1    19960514
AB   Proteins with domains derived from the binding region of an Ig heavy chain
     variable region and a binding region of an Ig light chain variable region
     that are linked but prevented from interacting to form an antigen binding
     site, associate to form antigen binding multimers, such as dimers, which may
     be multivalent or have multiple specificities.  The domains may be linked
     by a short peptide linker or may be joined directly together
     with bispecific dimers linked by longer linkers.  Methods of
     preparation of the polypeptides and multimers and of diverse repertoires of
     such proteins and their display on the surface of bacteriophage for easy
     selection of binders of interest, are described. along with many
     utilities.  The proteins have many anal., diagnostic, and therapeutic
     uses.  Modeling of Igs indicated that directly joining the VH
     antigen-binding domain to the VL antigen-binding domain would be possible
     with some structural constraints on higher-order structures but without
     the interaction of the two domains and so allow the generation of
     single-chain, bispecific antibodies (diabodies).  The invention
     is demonstrated by construction and characterization of a number of
     diabodies.  The genes for these antibodies were constructed by
     standard methods and expressed in Escherichia coli.


=> s scFv and linker and dimer and ((mammalian or animal) and cell)
L3          32 SCFV AND LINKER AND DIMER AND ((MAMMALIAN OR ANIMAL) AND CELL)

=>

=> duplicate remove L3
DUPLICATE PREFERENCE IS 'CAPLUS, BIOENG, BIOTECHNO, BIOTECHDS, ESBIOBASE'
KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
PROCESSING COMPLETED FOR L3
L4           24 DUPLICATE REMOVE L3 (8 DUPLICATES REMOVED)

=> d l4 bib abs 1-24

L4    ANSWER 1 OF 24  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN    2006-12793  BIOTECHDS
TI    Novel modified antibody comprising human immunoglobulin G-Fc and CDR
      region containing variable regions of light and heavy chain of
      anti-interleukin-6 receptor antibody as scFv, useful for
      treating rheumatoid arthritis and Crohn's disease;
         involving vector-mediated gene transfer and expression in human
         cell for use in rheumatoid arthritis, Castleman disease,
         atopic dermatitis, systemic lupus erythematosus, Crohn disease,
         pancreatic inflammation and psoriasis therapy and gene therapy
AU    CURIEL D T; PEREBOEV A; ADACHI Y; KISHIMOTO T; NISHIMOTO N
PA    UNIV OSAKA; UAB RES FOUND; CHUGAI SEIYAKU KK
PI    WO 2006046661 4 May 2006
AI    WO 2005-JP19820 27 Oct 2005
PRAI  US 2004-623018 28 Oct 2004; US 2004-623018 28 Oct 2004
DT    Patent
LA    Japanese
OS    WPI: 2006-332688 [34]
AN    2006-12793  BIOTECHDS
AB    DERWENT ABSTRACT:
      NOVELTY - A modified antibody (I) comprising scFv and Fc, in
      which the CDR region containing the variable regions of light chain and
      heavy chain of anti-interleukin(IL)-6 receptor antibody are expressed as
      a single strand as scFv and where the Fc region is derived from
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human IgG, is new.

DETAILED DESCRIPTION - A modified antibody (I) comprising scFv and Fc, in which the CDR region containing the variable regions of light chain and heavy chain are expressed as a single strand as scFv, and the Fc region is derived from human IgG, and where H-chain variable region has the amino acid sequence of CDR1: Ser-Asp-His-Ala-Trp-Ser (SEQ ID Number 3), CDR2: Tyr-Ile-Ser-Tyr-Ser-Gly-Ile-Thr-Thr-Tyr-Asn-Pro-Ser-Leu-Lys-Ser (SEQ ID Number 4), and CDR3: Ser-Leu-Ala-Arg-Thr-Thr-Ala-Met-Asp-Tyr (SEQ ID Number 5), and the L-chain has amino acid sequence of CDR1: Arg-Ala-Ser-Gln-Asp-Ile-Ser-Ser-Tyr-Leu-Asn (SEQ ID Number 6), CDR2: Tyr-Thr-Ser-Arg-Leu-His-Ser (SEQ ID Number 7), and CDR3: Gln-Gln-Gly-Asn-Thr-Leu-Pro-Tyr-Thr (SEQ ID Number 8). INDEPENDENT CLAIMS are also included for: (1) a pharmaceutical composition (C1), comprising (I) and a carrier; (2) producing (M1) a pharmaceutical composition, involves blending (I) with a carrier; (3) a polynucleotide (II) encoding (I); (4) a vector (V1) comprising (II); (5) a host cell (III) expressed with (II); (6) producing (I); (7) an IL-6 receptor inhibitor (IV) chosen from (I)-(III), as an active ingredient; (8) a vector (V2) for gene therapies, comprising (II) capable of expressing in a human cell; and (9) kit for treating the disease associated with IL-6, comprising a vector containing (II) capable of expressing in human cell, reagent for vector transduction and reagent for isolating peripheral blood lymphocyte.

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (III) in a medium, and recovering (I) from the culture (claimed). Preferred Antibody: (I) has H-chain variable region having a fully defined 118 amino acid (SEQ ID Number 1) sequence given in the specification. (I) has L-chain variable region having a fully defined 108 amino acid (SEQ ID Number 2) sequence given in the specification. The scFV is bound with the human IgGFc portion in the C-terminal of L-chain variable region. The scFV is bound to the N-terminal in the order of H-chain variable region, linker and L-chain variable region. The linker has amino acid sequence of Gly-Gly-Gly-Gly-Ser-Gly-Gly-Arg-Ala-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID Number 9). (I) is a dimer connected by a disulfide bond. Preferred Host Cell : (III) is of human cell origin.

ACTIVITY - Cytostatic; Antirheumatic; Antiarthritic; Dermatological; Antiinflammatory; Immunosuppressive; Gastrointestinal-Gen.; Antipsoriatic. No biological data given.

MECHANISM OF ACTION - IL-6 inhibitor (claimed); Gene therapy. The ability of the modified antibody to inhibit competitively the binding of IL-6 receptor and IL-6 was studied as follows. Modified anti-IL-6 antibody (10 ng/ml) was treated with sIL-6R on an enzyme linked immunosorbent assay plate. Results showed that inhibition was dose dependent.

USE - A polynucleotide (II) encoding (I) is useful for treating disease associated with IL-6, which involves transducing (II) capable of expressing in a human cell to a vector, and administering the cell transduced with the vector to the patient. The cell is collected from the patient under treatment. The cell is peripheral blood lymphocyte. IL-6 receptor inhibitor (IV) is useful for inhibiting IL-6 receptor (claimed). (I) is useful as an IL-6 inhibitor, useful in gene therapy, has anticancer activity. The disease associated with IL-6 is rheumatoid arthritis, Castleman's disease, atopic dermatitis, systemic lupus erythematosus, Crohn's disease, pancreatic inflammation and psoriasis.

ADMINISTRATION - (C1) is administered intramuscularly, subcutaneously, intracutaneously or intravenously, at a dose of 0.1-2000 (preferably 100-300) mg/day.

ADVANTAGE - (I) can be expressed as a single strand polypeptide, and thus can be expressed in vivo. (I) having a homo dimer structure of natural IgG can be generated efficiently.

EXAMPLE - The cDNA encoding the heavy chain and light chain of human

IL-6 receptor antibody were amplified by PCR. The VH gene fragment and VL gene fragment were connected by overlap extension PCR method, and the DNA fragment encoding the linker (Gly-Gly-Gly-Gly-Ser) (Gly-Gly-Arg-Ala-Ser) Gly-Gly-Gly-Gly-Ser-Ser) were introduced into pOPE101 bacteria expression vector. Then, NCoI and a NotI restriction enzyme region were introduced to 5' terminal and 3' terminal of DNA encoding scFv like equivalent, to produce pOPE101/scFv of anti-IL-6 receptor humanized antibody. The assembly of Fc/anti-IL-6 receptor humanized-antibody encoding DNA was performed to produce pSec Tag/Fc/scFv of anti-IL-6 receptor humanized antibody. Finally, the expression cassette containing CMV promoter and a BGH polyadenlyated signal was re-cloned to the pShuttle plasmid, to produce pShuttle/CMV/FC/scFv of anti-IL-6 receptor humanized antibody. The scFv section was spliced from pShuttle/CMV/Fc/scFv of human anti-IL-6 receptor antibody, and inserted in the EcoRV-SpeI region and the IL-6 inhibitor expression shuttle vector was perfected. An IL-6 inhibitor expression shuttle vector was cotransfected to Escherichia coli BJ5183 with pAdEasy-1. The virus genome was collected from the cell. Then, recombinant Ad virus genome was transfected into 293 cells, and cultured. Recombinant Ad5/IL-6 inhibitor was purified with the cesium chloride.(43 pages)

L4 ANSWER 2 OF 24 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2005-09844 BIOTECHDS
TI New non-naturally occurring single chain protein comprising polypeptides with binding domain, connecting regions and N-terminally truncated immunoglobulin, having immunological activity, useful for neutralizing infectious agent;
single chain protein production via plasmid expression in host cell for use in neutralization of bacterium
AU LEDBETTER J A; HAYDEN-LEDBETTER M S; THOMPSON P A
PA TRUBION PHARM INC
PI WO 2005017148 24 Feb 2005
AI WO 2003-US41600 24 Dec 2003
PRAI US 2003-627556 26 Jul 2003; US 2003-627556 26 Jul 2003
DT Patent
LA English
OS WPI: 2005-182370 [19]
AN 2005-09844 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A non-naturally occurring single chain protein comprising first polypeptide with a binding domain comprising heavy chain variable region, second polypeptide comprising a connecting region attached to first polypeptide, and a third polypeptide comprising N-terminally truncated immunoglobulin heavy chain constant region polypeptide, where non-naturally occurring single-chain protein is capable of immunological activity, is new.

DETAILED DESCRIPTION - A non-naturally occurring single chain protein (I) comprises a first polypeptide having a binding domain polypeptide capable of binding to a target molecule, the binding domain polypeptide comprising a heavy chain variable region, which comprises an amino acid substitution or deletion at one or more amino acid residues, a second polypeptide comprising a connecting region attached to the first polypeptide, and a third polypeptide comprising an N-terminally truncated immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, where the non-naturally occurring single-chain protein is capable of an immunological activity. INDEPENDENT CLAIMS are also included for the following: (1) reducing a target cell population in a subject, involves administering to the subject a protein that is less than 150 kD, which involves treating the target cell population with a first protein or peptide that binds to cells within the target cell population, and treating the target cell population with a second protein or peptide that capable of at least one of binding an Fc receptor, inducing target cell

apoptosis, or fix complements, where the first protein or peptide molecule is directly connected to the second protein or peptide molecule or, optionally, the first protein or peptide molecule and the second protein or peptide molecule are linked by a third protein or peptide molecule, and where the protein molecule is not an antibody, a member of the TNF family or the TNF receptor family, and is not conjugated with a bacterial toxin, a cytotoxic drug, or a radioisotope; (2) depleting (M1) cells in an animal, involves administering a modified IgE protein into the blood stream of an animal; (3) a polynucleotide (II) that encodes (I); (4) a cell containing (II); (5) a recombinant vector capable of expressing (I); (6) expressing (I) under conditions in which (I) is expressed; (7) a composition comprising (I) in combination with one or more additional therapeutic compounds; (8) displaying recombinant molecules, which molecules include a native or engineered immunoglobulin heavy chain variable region, the improvement comprising an immunoglobulin heavy chain region that includes one or more mutation, substitution, alteration, and/or deletion at one or more amino acid residue corresponding to positions 9, 10,11,12, 108, 110, and 112 in the heavy chain variable region; and (9) a non-naturally occurring single chain antigen-binding protein comprising protein having a mutation chosen from 2H7 scFv VH LI IS (CSC-S) H WCH2, WCH3, 2H7 scFv VH L11S IgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgE CH2 CH3 CH4, 2H7 scFv VH L11S mIgAH WIgACH2 T4CH3, 2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3, 2H7 scFv VH L11 S-(CSS-S) H K322S CH2 WCH3, 2H7 scFv VH L11S (SSS-S) IT P331S CH2 WCH3,2HQ scFv VH L11S (CSS-S) H P331S CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3, 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3, G28-1 scFv (SSS-S) H WCH2 WCH3, G28-1 scFv IgAH WCH2 WCH3, G28-1 scFv VH L11S (SSS-S) H WCH2 WCH3, 2H7 scFv IgAH IgAGH2CH3, 2H7 scFv IgAH IgAHCH2 T18CH3, 2Hand-40.2.220 scFv (SSS-S) H WCH2 WCH3 (bispecific anti-ccd20-anti-cd40) 2H7 scFv IgAH IgACH2 T4CH3-hCD89 TM/CT, GI9-4 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT, 2el2 scFv (CCC-P) WH WCH2 WCH3-hCD89 TM/CT, etc.

BIOTECHNOLOGY - Preferred Protein: In (I), the binding domain polypeptide comprising a heavy chain variable region comprising one or more amino acid deletion or substitution in positions 9, 10, 11, 12, 108, 110, 112 and the protein has an increased recombinant expression or stability relative to the protein not having an amino acid deletion or substitution. (I) is capable of binding to the target molecule, antibody dependent cell-mediated cytotoxicity and complement fixation, and is capable of decreasing the number of target cells. (I) has an increased expression or stability in mammalian cells relative to a protein not having the amino acid substitution. The second polypeptide comprises an N-terminally truncated IgE immunoglobulin heavy chain constant region polypeptide attached to the second polypeptide, where the non-naturally occurring single-chain protein is capable of an immunological activity. The connecting region comprises first, second, and third cysteine residues, where the first cysteine residue is N-terminal to the second cysteine and the second cysteine is N-terminal to the third cysteine, where one or both of the second and third cysteine residues is substituted or deleted, and where the non-naturally occurring single-chain protein is capable of an immunological activity. The amino acid deletion or substitution in the first polypeptide is at positions 12, 80, 81, 83, 105, 106, and 107. The connecting region comprises at lease a portion of an IgA hinge region. The connecting region attached to the first polypeptide, comprises three cysteine residues and one proline residue, where one or more of the cysteine residues is deleted or substituted. The binding domain polypeptide is a single chain Fv. The one or more amino acid substitution or deletion in the heavy chain variable region is effective to increase expression or stability of the protein relative to a protein without the

deletion or substitution. The binding domain polypeptide comprises an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide. (I) further comprises a second binding domain polypeptide capable of binding a second target molecule, the second binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide. The first target molecule and the second target molecule are different or same. The binding domain polypeptide is a single chain Fv comprising an amino acid substitution at position 11 in the heavy chain variable region. The amino acid substituted for the amino acid at position of 11 of the single chain Fv heavy chain variable region is selected from serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, and histidine. The leucine at position of 11 of the single chain Fv heavy chain variable region is replaced with an amino acid other than serine or by serine at position 11, and, where the protein is capable of antibody dependent cell-mediated cytotoxicity and complement fixation, and is capable of binding to the target molecule decreasing a number of target cells. The leucine is replaced by des-leucine at position-11. (I) has an increased recombinant expression or stability relative to the protein not having an amino acid substitution at position 11. The expression of the protein having an amino acid substitution at position 11 is 10-100 fold greater than the protein without a substitution at position 11. The expression is in mammalian cells. The binding domain polypeptide is a single chain Fv and the amino acid at position 11 of the heavy chain variable region of the single chain Fv has been deleted. The binding domain polypeptide is a single chain Fv and the binding domain polypeptide comprises a light chain variable region, where the light chain variable region has an amino acid deletion or substitution at one or more of amino acid positions 12, 80, 81, 83, 105, 106, and 107. The amino acid at position 106 has been substituted or deleted. The binding domain polypeptide binds to a tumor antigen. The binding domain polypeptide binds to an antigen on an immune effector cell. The binding domain polypeptide binds to a cancer cell antigen. The cancer cell antigen is a surface antigen. The cancer cell antigen is an intracellular antigen. The binding domain polypeptide binds to a B cell antigen. The B cell antigen is selected from CD19, CD20, CD22, CD37, CD40, CD80, and CD86. The single chain Fv binds to a B cell antigen. The single chain Fv is selected from HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, and 4.4.220 single chain Fv. The single chain Fv is selected from HD37 single chain Fv, 2H7 single chain Fv, G28-1 single chain Fv, FC2-2, UCHL-1, 5B9, L6, 10A8, 2e12, 40.2.36, G19-4, IDS, and 4.4.220 single chain Fv. The binding domain polypeptide is an scFv that binds to a B cell differentiation antigen. The binding domain polypeptide binds to a target selected from CD2, CD3, CD4, CD5, CD6, CDS, CD10, GDI Ib, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD28, CD30, CD37, CD40, CD43, CD50 (ICAM3), CD54 (ICAM1), CD56, CD69, CD80, CD86, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, L6, B7-H1, and HLA class II. The protein is capable of forming a complex comprising two or more of the proteins. The complex is a dimer or monomer. (I) is coupled to a drug, toxin, immunomodulator, polypeptide effector, isotope, label, or effector moiety. The immunological activity is selected from antibody dependent cell-mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin. The immunological activity comprises two immunological activities selected from antibody dependent cell -mediated cytotoxicity, complement fixation, induction of apoptosis, induction of one or more biologically active signals, induction of one or

more immune effector cells, activation of cellular differentiation, cellular activation, release of one or more biologically active molecules, and neutralization of an infectious agent or toxin. (I) is capable of induction of biologically active signals by activation or inhibition of one or more molecules selected from protein kinases, protein phosphatases, G-proteins, cyclic nucleotides or other second messengers, ion channels, and secretory pathway components, or which is capable of induction of one or more immune effector cells selected from NK cells, monocytes, macrophages, B cells , T cells, mast cells, neutrophils, eosinophils, and basophils. The induction of one or more immune effector cells leads to antibody dependent cell-mediated cytotoxicity or the release of one or more biologically active molecules. (I) is capable of cellular activation, where the activation leads to changes in cellular transcriptional activity. The cellular transcriptional activity is increased or decreased. The one or more biologically active molecules is a protease or cytokine. The cytokine is selected from moriokines, lymphokines, chemokines, growth factors, colony stimulating factors, interferons, and interleukins. (I) is capable of neutralization of an infectious agent, where the infectious agent is a bacterium, a virus, a parasite, or a fungus. (I) is capable of neutralization of a toxin, where the toxin is selected from endotoxins and exotoxins. The exotoxin is selected from anthrax toxin, cholera toxin, diphtheria toxin, pertussis toxin, Escherichia coli heat-labile toxin LT, E.coli heat stable toxin ST, shiga toxin Pseudomonas exotoxin A, botulinum toxin, tetanus toxin, Bordetella pertussis AC toxin, and Bacillus anthracis EF. The toxin is an endotoxin selected from saxitoxins, tetrodotoxin, mushroom toxins, aflatoxins, pyrrolizidine alkaloids, phytohemagglutinins, and grayanotoxins. (I) is capable of binding to an intracellular target to effect a cellular function. The binding domain polypeptide comprises a light chain variable region attached to the heavy chain variable region by a binding domain linker, where the binding domain linker comprises one or more peptide having a sequence Gly-Gly-Gly-Gly-Ser. (I) comprises three Gly-Gly-Gly-Gly-Ser peptides. The binding domain polypeptide comprises wild-type or engineered immunoglobulin variable region obtained from species selected from human, murine, rat, pig, and monkey. The binding domain polypeptide comprises a humanized immunoglobulin variable region. The N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CHS constant region polypeptide. The binding domain polypeptide is a single chain Fv that comprises at least a portion of a human constant region. The connecting region comprises a naturally occurring hinge region selected from a human hinge or its portion, human IgG hinge or its portion, human IgA hinge or its portion, human IgE hinge or its portion, camelid hinge region or its portion, IgG1 llama hinge region or its portion, nurse shark hinge region or its portion, and spotted ratfish hinge region or its portion. The connecting region preferably comprises a human IgE hinge or its portion. The connecting region comprises a human IgG1, IgG2, IgG3 or IgG4 hinge region having either zero or one cysteine residue. The connecting region comprises a human IgGA hinge region having between zero and two cysteine residues. The connecting region comprises a wild-type human IgG1 immunoglobulin hinge region. The connecting region comprises a glycosylation site. The connecting region has no cysteine residues capable of forming disulfide bonds or has one cysteine residue. The connecting region comprises a mutated wild-type immunoglobulin hinge region polypeptide comprising not more than, one cysteine residue. The connecting region is altered such that the protein has a reduced ability to dimerize. The connecting region comprises three cysteine residues and one proline residue, where one or more of the cysteine residues is deleted or substituted and the proline reside is substituted or deleted. The wild-type hinge region polypeptide is from human IgG1. The heavy chain constant region of the connecting region comprises CH2 and CH3 domains from IgG1, where proline is replaced by serine at position 331 in

the CH2 region, threonine is replaced by asparagines at position 256, lysine is replaced by glutamine at position 290, glutamic acid is replaced by lysine at position 258, and alanine is replaced by proline at position 339 in the CH2 region. Preferred Methods: In (M1), the modified IgE protein is administered or coadministered with a histamine release blocker. The connecting region comprises a IgG hinge or its portion, and the heavy chain constant region is from IgE and comprises CH3 and CH4 domains without a CH2 domain. The single chain protein comprises a single chain Fv binding domain from a 2e12, and the heavy chain constant region comprises IgE CH2, CH3, and CH4 domains, and where the heavy chain constant region is attached to a polypeptide comprising CD80 transmembrane and cytoplasmic tail domains.

ACTIVITY - Antibacterial; Virucide; Antiparasitic; Fungicide. No supporting data is given.

MECHANISM OF ACTION - None given.

USE - (I) is useful for neutralization of an infectious agent, where the infectious agent is a bacterium, a virus, a parasite, or a fungus (claimed).

EXAMPLE - No relevant example is given. (590 pages)

L4 ANSWER 3 OF 24 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2004:252106 CAPLUS
DN 140:269512
TI Multivalent and multispecific engineered antibodies
IN Holliger, Kaspar-philipp; Griffiths, Andrew David; Hoogenboom, Hendricus Renerus J. M.; Malmqvist, Magnus; Marks, James David; McGuinness, Brian Timothy; Pope, Anthony Richard; Prospero, Terence Derek; Winter, Gregory Paul
PA Medical Research Council, UK
SO U.S. Pat. Appl. Publ., 98 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004058400	A1	20040325	US 2002-247839	20020920
PRAI	US 2002-247839		20020920		

AB The authors disclose antibody constructs comprising a first heavy chain variable region and a second light chain variable region, the domains being linked but incapable of associating with each other to form an antigen binding site. These constructs associate to form antigen binding multimers, such as dimers, which may be multivalent or have multispecificity. The domains may be linked by a short peptide linker or may be joined directly together. Bispecific dimers may have longer linkers. Methods of preparation of the polypeptides and multimers and diverse repertoires thereof, and their display on the surface of bacteriophage for easy selection of binders of interest, are disclosed, along with many utilities.

L4 ANSWER 4 OF 24 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2004-10503 BIOTECHDS
TI New adzyme for enzymatically altering a substrate, useful for preparing a composition for treating diseases associated with a soluble or membrane bound molecule, e.g. allergic or inflammatory diseases;
involving vector-mediated gene transfer and expression in host cell for use in gene therapy
AU AFEYAN N B; BAYNES B; DASGUPTA R; LEE F D; WONG G G
PA COMPOUND THERAPEUTICS INC; AFEYAN N B
PI WO 2004019878 11 Mar 2004
AI WO 2003-US26937 27 Aug 2003
PRAI US 2002-430001 27 Nov 2002; US 2002-406517 27 Aug 2002
DT Patent
LA English

OS WPI: 2004-239110 [22]
AN 2004-10503 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An adzyme for enzymatically altering a substrate comprises a catalytic domain that catalyzes a chemical reaction converting the substrate to one or more products, and a targeting group that reversibly binds with an address site on the substrate or with an address site on a second molecule that occurs in functional proximity to the substrate (the targeting moiety and the catalytic domain are heterologous with respect to each other).

DETAILED DESCRIPTION - An adzyme for enzymatically altering a substrate comprises a catalytic domain that catalyzes a chemical reaction converting the substrate to one or more products, and a targeting moiety that reversibly binds with an address site on the substrate or with an address site on a second molecule that occurs in functional proximity to the substrate, where the targeting moiety and the catalytic domain are heterologous with respect to each other, the targeting moiety, when provided separately, binds to the substrate, the catalytic domain, when provided separately, catalyzes the chemical reaction converting the substrate to one or more products. The adzyme has one or more properties, with respect to the reaction with the substrate, of: (a) a potency at least 2 times greater than the catalytic domain or the targeting moiety alone; (b) a kon of 103 M-s-1 or greater; (c) a kcat of 0.1 sec-1 or is greater; (d) a KD that is at least 5 fold less than the Km of the catalytic domain; (e) a k0ff of 10-4 sec-1 or greater; (f) a catalytic efficiency at least 5 fold greater than the catalytic efficiency of the catalytic domain alone; (g) a Km at least 5 fold less than the Km of the catalytic domain alone; and/or (h) an effective substrate concentration that is at least 5 fold greater than the actual substrate concentration. INDEPENDENT CLAIMS are also included for: (1) an adzyme preparation for therapeutic use in a human patient comprising the adzyme; (2) a method of making a medicament for use in treating a disorder that is associated with an activity of the substrate of the adzyme; (3) a method of making a medicament for use in treating an inflammatory or allergic disorder; (4) a method of treating a disorder that is associated with an activity of the substrate of the adzyme; (5) a method of treating an inflammatory of allergic disorder; (6) a nucleic acid comprising a coding sequence for the adzyme; (7) an expression vector comprising the nucleic acid; (8) a cell comprising the expression vector; (9) a method for manufacturing an adzyme; (10) a method of designing and constructing an effective adzyme; and (11) a method of operating a therapeutic adzyme business.

BIOTECHNOLOGY - Preferred Adzyme: The adzyme comprises a catalytic domain that catalyzes a chemical reaction converting the substrate to one or more products, and a targeting moiety that reversibly binds with an address site on the substrate or with an address site on a second molecule that occurs in functional proximity to the substrate, where the substrate is an extracellular signaling molecule, the targeting moiety and the catalytic domain are heterologous with respect to each other, the targeting moiety, when provided separately, binds to the substrate, the catalytic domain, when provided separately, catalyzes the chemical reaction converting the substrate to one or more products. The adzyme is more potent than the catalytic domain or targeting moiety with respect to the reaction with the substrate. The adzyme comprises a polypeptide comprising a catalytic domain that catalyzes a chemical reaction converting the substrate to one or more products, a targeting domain that reversibly binds with an address site on the substrate or with an address site on a second molecule that occurs in functional proximity to the substrate, and a linker joining the catalytic domain and the targeting domain, where the substrate is an extracellular polypeptide signaling molecule, the targeting moiety and the catalytic domain are heterologous with respect to each other, the targeting domain, when provided separately, binds to the substrate, the catalytic domain, when provided separately, catalyzes the chemical reaction converting the

substrate to one or more products, and the adzyme is more potent than the catalytic domain or targeting moiety with respect to the reaction with the substrate. The substrate is endogenous to a human patient. The adzyme comprises a polypeptide comprising a catalytic domain that catalyzes a chemical reaction converting the substrate to one or more products, a targeting domain that reversibly binds with an address site on the substrate or with an address site on a second molecule that occurs in functional proximity to the substrate, and a linker joining the catalytic domain and the targeting domain, where the substrate is an extracellular polypeptide signaling molecule, the targeting moiety and the catalytic domain are heterologous with respect to each other, the targeting domain, when provided separately, binds to the substrate, the catalytic domain, when provided separately, catalyzes the chemical reaction converting the substrate to one or more products, and the adzyme is more potent than the catalytic domain or targeting moiety with respect to the reaction with the substrate. The substrate is endogenous to a human patient. The effect of the adzyme on the substrate is effective against the target molecule in the presence of physiological levels of an abundant human serum protein. The abundant human serum protein is human serum albumin. The adzyme is a fusion protein. The fusion protein includes a linker between the catalytic domain and the targeting moiety. The linker is an unstructured peptide. The linker includes one or more repeats of Ser4Gly or SerGly4. The linker is selected to provide steric geometry between the catalytic domain and the targeting moiety such that the adzyme is more potent than the catalytic domain or targeting moiety with respect to the reaction with the substrate. The linker is selected to provide steric geometry between the catalytic domain and the targeting moiety such that the address moiety presents the substrate to the enzymatic domain at an effective concentration at least 5 fold greater than would be present in the absence of the address moiety. The he fusion protein is a cotranslational fusion protein encoded by a recombinant nucleic acid. The substrate is a biomolecule produced by a cell. The substrate is a polypeptide, a polysaccharide, a nucleic acid, a lipid or a small molecule. The substrate is a diffusible extracellular molecule. The diffusible extracellular molecule is an extracellular signaling molecule. The extracellular signaling molecule is an extracellular polypeptide-signaling molecule. The extracellular signaling molecule is interleukin-1 or TNF-alpha. The extracellular signaling molecule binds to a cell surface receptor and triggers receptor-mediated cellular signaling. The substrate is a receptor. The substrate is a unique receptor subunit of a heteromeric receptor complex. The biomolecule is a component of a biomolecular accretion. The biomolecular accretion is an amyloid deposit and an atherosclerotic plaque. The biomolecule is a biomolecule produced by a pathogen. The pathogen is a protozoan, fungus, bacterium or virus. The biomolecule is a prion protein. The substrate comprises a polypeptide and where the catalytic domain is a protease that cleaves at least one peptide bond of the substrate. The adzyme is resistant to cleavage by the catalytic domain. The catalytic domain is a protease that cleaves at least one peptide bond of the substrate. The adzyme is resistant to cleavage by the catalytic domain. The protease is a zymogen. The adzyme is purified from a cell culture in the presence of a reversible protease inhibitor. The adzyme is purified from a cell culture in the presence of a reversible protease inhibitor. The catalytic domain modifies one or more pendant groups of the substrate. The substrate includes a chiral atom, and the catalytic domain alters the ratio of stereoisomers. The catalytic domain alters the level of post-translational modification of the polypeptide substrate. The catalytic domain alters the level of post-translational modification of the polypeptide substrate. The post-translation modification is glycosylation, phosphorylation, sulfation, fatty acid modification, alkylation, prenylation or acylation. The post-translation modification is glycosylation, phosphorylation, sulfation, fatty acid modification, alkylation, prenylation or acylation. The catalytic domain is a protease,

an esterase, an amidase, a lactamase, a cellulase, an oxidase, an oxidoreductase, a reductase, a transferase, a hydrolase, an isomerase, a ligase, a lipase, a phospholipase, a phosphatase, a kinase, a sulfatase, a lysozyme, a glycosidase, a nuclease, an aldolase, a ketolase, a Iyase, a cyclase, a reverse transcriptase, a hyaluronidase, an amylase, a cerebrosidase or a chitinase. The catalytic domain is a protease, an oxidase, an oxidoreductase, a reductase, a transferase, a hydrolase, an isomerase, a lipase, a phospholipase, a phosphatase, a kinase, a sulfatase, a glycosidase, an aldolase, a ketolase, a lyase, a cyclase, a hyaluronidase or a cerebrosidase. The adzyme is resistant to autocatalysis. The adzyme is resistant to autocatalysis at an adzyme concentration that is about equal to the concentration of adzyme in a solution to be administered to a subject. The adzyme alters the half-life of the biomolecule in vivo. The adzyme alters the distribution of the biomolecule or substrate in vivo. The adzyme reduces a biological activity of the biomolecule. The biomolecule binds different molecules in vivo, and the adzyme alters the binding specificity of the biomolecule. The substrate binds different molecules in vivo, and the adzyme alters the binding specificity of the substrate. The adzyme alters the interaction of the biomolecule or substrate with other molecules in vivo. The adzyme alters one or more of: a receptor-ligand interaction, a protein-protein interaction or a DNA-protein interaction. The adzyme reduces receptor-mediated or ion channel-mediated signal transduction. The adzyme alters proliferation, differentiation or viability of a cell in vivo or in vitro. The product of the chemical reaction is an antagonist of the substrate. The adzyme alters an intrinsic enzymatic activity of the biomolecule. The substrate is a polypeptide. The polypeptide is present in biological fluid of an animal. The extracellular polypeptide-signaling molecule is present in biological fluid of an animal. The biological fluid is blood or lymph. The polypeptide substrate is a polypeptide hormone, a growth factor and/or a cytokine. The polypeptide factor is four-helix bundle factors, EGF-like factors, insulin-like factors, -trefoil factors and cysteine knot factors. The extracellular polypeptide-signaling molecule is selected from the group consisting of four-helix bundle factors, EGF-like factors, insulin-like factors or cysteine knot factors. The polypeptide is a pro-inflammation mediator and the enzyme construct reduces the pro-inflammatory activity of the polypeptide factor. The extracellular polypeptide-signaling molecule is a pro-inflammation mediator and the enzyme construct reduces the pro-inflammatory activity of the polypeptide factor. The adzyme further comprises a transcytosis moiety that promotes transcytosis of the adzyme into the cell. The targeting moiety comprises a polypeptide or polypeptide complex. The targeting moiety is a polyanionic or polycatonic-binding agent. The targeting moiety is an oligonucleotide, a polysaccharide or a lectin. The targeting moiety is an antibody or polypeptides. The targeting moiety is a monoclonal antibody, an Fab and F(ab)2, an scFv, a heavy chain variable region or a light chain variable region. The substrate is receptor ligand, and the targeting moiety includes a ligand-binding domain of a cognate receptor of the ligand. The targeting moiety is an artificial protein or peptide sequence engineered to bind to the substrate. The substrate is a receptor, and the targeting moiety is a cognate ligand of the receptor. The targeting moiety is a cognate ligand of the receptor. The substrate is TNFa and where the targeting moiety binds to TNFa. The catalytic domain comprises a protease that decreases TNFa pro-apoptotic activity. The catalytic domain comprises a protease that decreases TNFa pro-apoptotic activity. The protease is MT1-MMP, MMP12, tryptase, MT2-MMP, elastase, MMP7, chymotrypsin, or trypsin. The targeting moiety is an sp55 portion of TNFR1. The substrate is IL-I and the targeting moiety binds to IL-1. The substrate is IL-I and where the targeting moiety binds to IL-I. The catalytic domain comprises a protease that decreases an IL-I bioactivity. The catalytic domain comprises a protease that decreases an IL-I bioactivity. Preferred Vector: The expression vector directs expression of the adzyme in a suitable host cell

. It comprises the nucleic acid. Preferred Cell: The cell comprises a first nucleic acid comprising a first coding sequence and a second nucleic acid comprising a second coding sequence, where the first coding sequence encodes a first fusion protein comprising an immunoglobulin heavy chain and a catalytic domain, and where the second coding sequence encodes a second fusion protein comprising an immunoglobulin heavy chain and a targeting domain. The cell secretes an adzyme comprising an Fc fusion protein construct that is a dimer of the first fusion protein and the second fusion protein. Preferred Preparation: The adzyme preparation further comprises a carrier. The adzyme preparation is formulated such that autocatalytic modification of the adzyme is inhibited. The adzyme preparation comprises a catalytic domain that is a protease. The adzyme preparation further comprises a reversible inhibitor of the protease. The reversible inhibitor is safe for administration to a human patient. The adzyme preparation is substantially pyrogen free. The adzyme preparation is packaged with instructions for administration to a patient. Preferred Method: Making a medicament for use in treating a disorder that is associated with an activity of the substrate of the adzyme comprises formulating the adzyme for administration to a human patient. Making a medicament for use in treating an inflammatory or allergic disorder comprises formulating the adzyme for administration to a human patient, where the substrate of the adzyme is an inflammatory cytokine. Treating a disorder that is associated with an activity of the substrate of the adzyme comprises administering the adzyme to a human patient. Treating an inflammatory of allergic disorder comprises administering the adzyme to a human patient, where the substrate of the adzyme is an inflammatory cytokine. Manufacturing an adzyme comprises culturing a cell in conditions that cause the cell to produce the adzyme encoded by the expression vector and purifying the adzyme to substantial purity. Purifying the adzyme to substantial purity includes the use of a reversible inhibitor that inhibits autocatalytic activity of the catalytic domain. The catalytic domain of the adzyme is a protease domain, and purifying the adzyme to substantial purity includes the use of a reversible protease inhibitor that inhibits the protease activity of the catalytic domain. Designing and constructing an effective adzyme comprises: (a) selecting a substrate that is a known target for a therapeutically effective binding agent; (b) testing catalytic domains for effectiveness in reducing an activity of the substrate to obtain a set of one or more candidate catalytic domains that are effective in reducing an activity of the substrate; (c) testing binding moieties for effectiveness in binding to the substrate to obtain a set of one or more candidate targeting moieties that are effective in binding to the substrate; (d) constructing and producing adzymes comprising one or more of the candidate catalytic domains and one or more of the candidate targeting moieties, where the one or more catalytic domains and the one or more candidate targeting moieties are associated in at least two different geometric conformations, testing the plurality of adzymes for effectiveness in reducing an activity of the substrate to obtain a set of one or more candidate adzymes, where an adzyme that is effective for reducing an activity of the substrate is an effective adzyme. The method further comprises testing the efficacy of the adzyme in an organism and modifying an effective adzyme to improve one or more of the following properties: (a) reduce the amount of autocatalysis; (b) increase the potency of the adzyme; (c) increase the specificity of the adzyme; (d) improve the balance of the potency and specificity of the adzyme; (e) increase the serum half-life of the adzyme; and (f) decrease the interactions between the adzyme and one or more abundant serum proteins. Operating a therapeutic adzyme business comprises: (a) designing an adzyme; (b) testing the adzyme for safety and effectiveness in humans; and (c) arranging for distribution and marketing of the adzyme.

ACTIVITY - Antiallergic; Antiinflammatory. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The adzyme is useful for preparing a composition for treating

diseases associated with a soluble or membrane bound molecule, e.g., allergic or inflammatory disease.
EXAMPLE - No relevant examples given.(122 pages)

L4 ANSWER 5 OF 24 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2004:1086657 CAPLUS
DN 142:91800
TI Bispecific minibodies targeting HER2/neu and CD16 exhibit improved tumor lysis when placed in a divalent tumor antigen binding format
AU Shahied, Lillian S.; Tang, Yong; Alpaugh, R. Katherine; Somer, Robert; Greenspon, Dana; Weiner, Louis M.
CS Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA, 19111, USA
SO Journal of Biological Chemistry (2004), 279(52), 53907-53914
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB Unconjugated monoclonal antibodies have emerged as important therapeutic agents for selected malignancies. One mechanism by which antibodies can exert cytotoxic effects is antibody-dependent cellular cytotoxicity (ADCC). In an effort to increase the efficiency of ADCC at tumor sites, the authors have focused on the construction of bispecific antibodies specific for the tumor antigen HER2/neu and the FcγRIII-activating receptor (CD16) found on NK cells, mononuclear phagocytes, and neutrophils. Here, the authors describe the production of bispecific minibodies in two distinct binding formats. The parent minibody was constructed such that the IgG1 CH3 constant domain serves as the oligomerization domain and is attached to an anti-CD16 and an anti-HER2/neu single-chain Fv via 19- and 29-amino acid linkers, resp. This mol. can be expressed in mammalian cells from a dicistronic vector and has been purified using sequential affinity purification techniques. Anal. by surface plasmon resonance shows that the bispecific minibody can bind to HER2/neu and CD16, both individually and simultaneously. Furthermore, cytotoxicity studies show that the minibody can induce significant tumor cell lysis at a concentration as low as 20 nM. A trimeric, bispecific minibody (TriBi) that binds dimerically to HER2/neu and monomerically to CD16 induces equivalent cytotoxicity at lower antibody concns. than either the parent minibody or the corresponding single-chain dimer. Both minibody constructs are stable in mouse and human serum for up to 72 h at 37°. These minibodies have the potential to target solid tumors and promote tumor lysis by natural killer cells and mononuclear phagocytes.
RE.CNT 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 24 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2004:96700 CAPLUS
DN 140:211581
TI Selection of genetically modified cell population using hapten-specific antibody/receptor chimera
AU Kawahara, Masahiro; Kimura, Hiroko; Ueda, Hiroshi; Nagamune, Teruyuki
CS Graduate School of Engineering, Department of Chemistry and Biotechnology, The University of Tokyo, Bunkyo-ku, Tokyo, 113-8656, Japan
SO Biochemical and Biophysical Research Communications (2004), 315(1), 132-138
CODEN: BBRCA9; ISSN: 0006-291X
PB Elsevier Science
DT Journal
LA English
AB Efficient selection of the genetically modified cell population is a critical step to obtain the cells with desired properties. In this study, the authors propose an antigen-mediated genetically modified cell amplification (AMEGA) system employing an antibody/receptor

chimera that triggers a growth signal in response to a non-toxic hapten dimer. An anti-fluorescein single-chain Fv fused to the extracellular D2 domain of erythropoietin receptor and transmembrane/intracellular domains of gp130 was expressed together with a model transgene, enhanced green fluorescent protein (EGFP) downstream of IRES sequence, by retroviral infection to IL-3-dependent Ba/F3 cells. Addition of fluorescein dimers connected by various oligo-DNA linkers induced selective growth of transfectants, thus leading to efficient expansion of EGFP-pos. cell population. Also, digestion of the oligonucleotides by specific restriction endonuclease completely suppressed cell growth. Because these hapten dimers are not harmful for normal cells, the approach will be especially useful for reversible in vitro or in vivo expansion of genetically modified cell population employed for cell therapy and tissue engineering.

RE.CNT 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 7 OF 24 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2004-08884 BIOTECHDS
TI Immunosuppressive properties of anti-CD3 single-chain Fv and diabody; antibody production via cell culture against CD3 for use in therapy
AU LE GALL F; REUSCH U; MOLDENHAUER G; LITTLE M; KIPRIYANOV SM
CS Affimed Therapeut AG; German Canc Res Ctr
LO Kipriyanov SM, Affimed Therapeut AG, Neuenheimer Feld 582, D-69120 Heidelberg, Germany
SO JOURNAL OF IMMUNOLOGICAL METHODS; (2004) 285, 1, 111-127 ISSN: 0022-1759
DT Journal
LA English
AN 2004-08884 BIOTECHDS
AB AUTHOR ABSTRACT - The mouse anti-human CD3 monoclonal antibody OKT3 is a potent immunosuppressive agent used in clinical transplantation. However, OKT3 therapy is associated with unpleasant and often serious side effects which appear to result from cytokine release, complement activation and a human anti-mouse antibody (HAMA) response. To decrease these adverse side effects, we constructed antibody fragments comprising OKT3 variable domains without any constant domains. Single-chain Fv (scFv) monomers, dimers and trimers were generated by changing the linker length between the V-H and V-L domains. The linkers used were the natural extensions of the V-H into the C(H)1 domain. The dimeric molecules (diabodies) demonstrated the best CD3-binding activity. The diabody with the six amino acid linker was produced in bacteria with a tenfold higher yield than other scFvs and possessed CD3-binding affinity approaching that of the parental mAb. In contrast to OKT3 mAb, the anti-CD3 diabody and scFv monomer did not cause any T-cell activation and cytokine release in vitro, while demonstrating CD3 modulation. In mixed lymphocyte cultures, both diabody and scFv, but not the monoclonal antibody OKT3, were able to suppress T-cell activation and secretion of IL-2 and IFN-gamma in a dose-dependent manner. The anti-CD3 diabody may provide a potent immunosuppressive drug with low toxicity and immunogenicity. (C) 2004 Elsevier B.V. All rights reserved.
DERWENT ABSTRACT: For antibody production, Escherichia coli K12 strain RV308 was used for functional expression of antibody fragments. The bacteria transformed with the expression plasmids were grown in shaking flasks and induced essentially as described. To isolate soluble periplasmic proteins, the pelleted bacteria were resuspended in 5% of the initial volume of ice-cold 200 mM Tris-HCl, 20% sucrose, 1 mM EDTA, pH 8.0. After 1 hr incubation on ice with occasional stirring, the spheroplasts were pelleted by centrifugation and the supernatant containing the soluble periplasmic proteins was thoroughly dialyzed

against 50 mM Tris-HCl, 1 M NaCl, pH 7.0. The recombinant product was concentrated by ammonium sulfate precipitation. The protein precipitate was collected by centrifugation and dissolved in 10% of the initial volume of 50 mM Tris-HCl, 1 M NaCl, pH 7.0, followed by thorough dialysis against the same buffer. Immobilized metal affinity chromatography (IMAC) was performed at 4 deg using a 5-ml column of Chelating Sepharose charged with Cu 2+ and equilibrated with 50 mM Tris-HCI, 1 M NaCl, pH 7.0. The sample was loaded by passing the sample over the column by gravity flow. The column was then washed with 20 column volumes of start buffer followed by start buffer containing 50 mM imidazole until the absorbance of the effluent was minimal. Absorbed material was eluted with 50 mM Tris-HCI, 1 M NaCl, 300 mM imidazole, pH 7.0, as 1 ml fractions. The eluted fractions containing recombinant protein were identified by reducing 12% SDS-PAGE followed by Coomassie staining. The final purification of scFv was achieved by ion-exchange chromatography on a Mono S HR5/5 column(17 pages)

L4 ANSWER 8 OF 24 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2003-17622 BIOTECHDS
TI New bispecific antibody, useful for inducing T cell mediated destruction of tumor cells, has binding sites for tumor antigen and CD28 T cell receptor;
antibody production via plasmid expression in host cell for use in disease therapy
AU JUNG G; JUNG G
PA JUNG G; JUNG G
PI WO 2003042231 22 May 2003
AI WO 2002-EP12545 9 Nov 2002
PRAI DE 2001-1056482 12 Nov 2001; DE 2001-1056482 12 Nov 2001
DT Patent
LA German
OS WPI: 2003-457489 [43]
AN 2003-17622 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A bispecific antibody (A) comprising one binding site for the T cell receptor CD28 and a second for a tumor antigen (TAg), where each binding site comprises the variable domains of the light and heavy chains and the heavy domains are connected through a peptide linker, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) bispecific antibodies (A') that are specific for CD28 and TAg and bivalent at least for CD28; (2) a nucleic acid (I) that encodes (A) and (A'); (3) a vector that contains (I); and (4) cells that contain (I) or the vector of (3).

BIOTECHNOLOGY - Preparation: The antibodies are prepared by standard recombinant techniques. Preferred Antibodies: The peptide linker includes at least part of the N-terminus of the CH1 domain of human immunoglobulin G (IgG), and at least part of the fos-jun adapter (or hinge region) is fused to one light chain. In (A') both of the constant domains of a Fab fragment specific for a tumor are fused to a single-chain Fv antibody (scFv) fragment specific for CD28. Optionally (A') is also bivalent for TAg and then consists of two (A) or has a scFv specific for CD28 fused to both heavy chains of a complete anti-TAg antibody. (A) dimerizes spontaneously, creating a dimer with two binding sites for each antigen. TAg is melanoma-associated proteoglycan, HER-2/neu or CD20. Preferred Cells: The cells of (4) are mammalian, insect, bacterial, plant or yeast cells.

ACTIVITY - Cytostatic. Melanoma cells were incubated for 4 days with peripheral blood mononuclear cells and 200 mg of a bispecific antibody that recognized both CD28 and melanoma-associated proteoglycan and a dimer of the antibody. Tumor cell destruction was about 40% for the antibody in monomer form and nearly 70% for the antibody in dimer form.

MECHANISM OF ACTION - T-cell Activator. Particularly no stimulation of CTLA-4 on activated T cells is required, so proliferation of T cells is not limited.

USE - (A) or (A') are useful for treating cells to effect supra-agonistic tumor cell-induced activation of T cells such that no additional exogenous stimulus is required. In the method, TAg-expressing cells are incubated with T cells and (I), and are then selectively killed (claimed). (A) are useful for the treatment and prevention of tumors.

EXAMPLE - A sequence encoding a monospecific single-chain Fv fragment (scFv, Vl-FL-Vh) was prepared comprising the heavy and light chain variable domains (Vh, Vl) connected through (Gly4Ser)3, then attached to a linker (L) representing the N-terminus of the human immunoglobulin G CH1 region. A construct of structure (Vl-FL-Vh)9.3-L-(Vh-FL-Vh)9.2.27-6His (9.3 refers to a CD28-specific antibody and 9.2.27 to an antibody specific for the melanoma-associated proteoglycan) was then prepared and coupled at the 5'-end to a promoter and at the 3'-end to an intron, containing an enhancer, and a polyA tail. This construct was cloned into a pcDNA3/pCR-Script vector fusion and the recombinant plasmid used to transform J558 melanoma cells. The recombinant antibodies produced formed dimers spontaneously.
(33 pages)

L4 ANSWER 9 OF 24 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2003-18917 BIOTECHDS
TI Novel polypeptide antigen which includes epitope overexpressed by tumor cells e.g. B-cell lymphoma, and is capable of inducing immune response in mammal without need for adjuvant, useful as anti-tumor vaccine component;
single chain antibody expression in transgenic plant for use as a recombinant vaccine against cancer
AU MCCORMICK A A; TUSE D; REINL S J; LINDBO J A; TURPEN T H
PA MCCORMICK A A; TUSE D; REINL S J; LINDBO J A; TURPEN T H
PI US 2003039659 27 Feb 2003
AI US 2002-67892 8 Feb 2002
PRAI US 2002-67892 8 Feb 2002; US 1999-155979 24 Sep 1999
DT Patent
LA English
OS WPI: 2003-492153 [46]
AN 2003-18917 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A polypeptide self-antigen (I) useful as tumor- specific vaccine in subject with a tumor, including an epitope or epitope unique to, or overexpressed by, cells of the tumor, is produced in a cell or organism that has been transformed or transfected with the nucleic acid derived from tumor of subject, and is capable of inducing an immune response in a mammal without a need for adjuvant or other immunostimulatory materials, is new.

DETAILED DESCRIPTION - A polypeptide self-antigen (I) useful as a tumor-specific vaccine in a subject with a tumor or at risk of developing a tumor, encoded at least in part by a nucleic acid in the cells of the tumor, is new. The polypeptide: (a) includes an epitope or epitope unique to, or overexpressed by, cells of the tumor, thus distinguishing the tumor from all other tumors of the same or different histological type, in the subject or in another member of the subject's species; (b) is produced in a cell or organism that has been transformed or transfected with the nucleic acid derived from the tumor of the subject; (c) is obtainable from the cell or organism in correctly folder from, without a need for denaturation and renaturation and mimics the epitope or epitopes in their native form; or (d) is capable of inducing an immune response in a mammal, including the subject, without a need for adjuvant or other immunostimulatory materials, so that administration of the polypeptide results in an antibody or cell-mediated immune response to the epitope or

epitopes. INDEPENDENT CLAIMS are also included for the following: (1) an individual-specific immunogenic product (II) comprising (I) produced transiently in a plant, and which is a 2-domain scFv antibody that includes part of variable heavy (VH) and variable light (VL) domains and are linked by an amino acid linker, comprising: (a) joining a nucleic acid encoding the first domain of the polypeptide to a nucleic acid encoding a first part of a linker to produce a first nucleic acid construct; (b) joining the nucleic acid encoding a second part of the linker to a nucleic acid encoding the second domain of the polypeptide to produce a second nucleic acid construct; (c) incorporating the first and second constructs into a transient plant expression vector in frame so that, when expressed, the polypeptide bears the first and second domain separated by the linker, transfecting a plant with the vector so that the plant transiently produces the polypeptide; and (d) recovering the polypeptide as a soluble, correctly-folded protein; (2) a vaccine composition (III) useful for inducing a tumor specific immune response, e.g. a idiotype-specific anti-lymphoma immune response, comprising (I) produced transiently in a plant, and which is a 2-domain scFv antibody that includes part of VH and VL domains and are linked by an amino acid linker, and a carrier or excipient; (3) a vaccine composition (IV) useful for inducing a polyclonal immune response to an idiotype in a mouse comprising (II) and a carrier or excipient; and (4) producing (I).

BIOTECHNOLOGY - Preparation: Producing (I) which is a 2- domain scFv antibody that includes part of VH and VL domains and are linked by an amino acid linker, involves carrying out the method of (1). Preferred Polypeptide: (I) is produced in a plant, preferably transiently in the transformed or transfected plant. (I) comprises at least two peptide domains. The tumor is a B-cell lymphoma and the tumor epitope is a surface immunoglobulin epitope, where (I) includes at least one idiotypic epitope of the V region of the immunoglobulin, and more preferably comprises two V region domains of the immunoglobulin. The two domains are at least part of the VH and at least part of the VL domains of the immunoglobulin. The part of the VH region includes at least one complementarity-determining region (CDR), preferably CDR2. Preferably, (I) is a two-domain single chain antibody (scFv) that includes at least part of the VH and the VL domains, and more preferably includes the VH and VL domains, where the domains are linked by an amino acid linker that has between 1-50 residues, consists of between 1-12 different amino acids, and facilitates secretion and correct folding of the polypeptide to mimic the tumor epitope in its native form in or on the tumor cell. The linker is a member of a randomized library of linkers that vary in size and sequence, and the library is encoded by nucleic acid sequences consisting of a repeated pattern of degenerate repeated triplet nucleotides having the following requirements. Position 1 of each repeated triplet cannot be the same nucleotide as position 2 or position 3 of the repeated triplet, or position 2 of each repeated triplet cannot be the same nucleotide as position 3 of the repeated of triplet. The nucleotide in the first and second positions of each repeated triplet is chosen from any two of deoxyadenosine, deoxyguanosine, deoxycytidine or deoxythymidine, where the position 1 of each repeated triplet is deoxyadenosine or deoxyguanosine, position 2 of each repeated triplet is deoxycytidine or deoxyguanosine, and position 3 of each repeated triplet is deoxythymidine. The two domain scFv polypeptide is in solution, is adsorbed to, bound to, or integrated into, a carrier or delivery system. The polypeptide upon administration to a mammalian host (preferably human), including the subject induces a polyclonal anti-idiotypic antibody response or a cell-mediated immune response. The polyclonal anti-idiotypic responses are detected by testing serum or peripheral blood cells of the host. The antibody response is measured in an enzyme immunoassay or by flow cytometry. Preferred Product: (II) is a scFv polypeptide where the first domain is the Ig VH domain and the second domain is Ig VL domain, both of

which domains create an idiotype of the immunoglobulin of the B cell lymphoma, and where the product induces an idiotype-specific antibody or cell-mediated immune response directed to the lymphoma upon administration to a subject. The plant is a plant cell. (II) is in aqueous solution, or is adsorbed to, bound, or integrated into, a carrier or delivery system. Preferred Vaccine Composition: (III) comprises a polypeptide that is a scFv including the VH and the VL domains. The vaccine when administered to the subject which the tumor originated, elicits a protective anti-tumor immune response which is a polyclonal anti- idiotypic antibody response against idiotope of an idiotype of a surface immunoglobulin, or a T-cell mediated anti-idiotypic response. The vaccine composition further comprises an immunostimulatory cytokine (interleukin-1 (IL-1), IL-12, IL-18 or interferon-gamma) or chemokine. (III) in unit dosage form includes 0.1-10 mg of the polypeptide, and the excipient is sterile saline.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Inducer of protective anti-tumor immune response (cellular, humoral or both) in a mammal; Vaccine. An idiotype-bearing scFv was produced from lymphoma cells of a human subject (designated JJ) using mRNA from the lymphoma cells to make cDNA which is PCR amplified using appropriate primers to amplify the VH and VL coding sequences. This DNA was expressed in a Nicotiana benthamiana plant by cloning into modified tobamoviral vector using the random linker library approach. The scFv corresponding to JJ's lymphoma surface Ig idiotype was obtained from the plants and formulated into a vaccine. The vaccine was administered by successive SC injections of 0.5 mg of the antigen. JJ's response was evaluated by laboratory tests and clinical observation. The following results were obtained. JJ's serum contained antibodies specific for the vaccine immunogen and reactive with a monoclonal Ig (that corresponds to the idiotypic lymphoma surface Ig). JJ's peripheral blood T lymphocytes responded significantly in vitro to the vaccine polypeptide (or to the lymphoma cells as stimulators) by proliferation, measured as 3H-thymidine incorporation and by secretion of interferon-gamma. JJ's peripheral blood mononuclear cells also produce tumor necrosis factor (TNF)-alpha in response to these stimuli. JJ's clinical response was characterized by radiographic evidence of lack of tumor progression and gradual disappearance of the lymphoma.

USE - (I) is useful for inducing an immune response, preferably a protective anti-tumor immune response in a mammal, preferably human. (III) is useful for inducing a tumor-specific immune antibody response in a tumor-bearing subject (preferably human) or a subject who had a tumor and was treated so that no tumor is clinically radiographically evident. (III) comprises the polypeptide in unit dosage form in aqueous solution at a concentration of 0.1-10 mg/ml. The vaccines are preferably useful for inducing immune antibody response against B-cell lymphoma. (All claimed.)

ADMINISTRATION - At least 15 microg of (I) is administered three times about two weeks apart by subcutaneous immunization. (III) is administered by parenteral route e.g. subcutaneous, transdermal or intramuscular route. (All claimed.)

ADVANTAGE - The polypeptide is produced without the need for denaturation or renaturation. (I) is rapidly produced in plants by transient viral expression. Plant samples expressing the desired protein can be positively identified by both enzyme linked immunosorbent assay (ELISA) and Western blotting 4 weeks after molecular cloning. Thus, (I) is expressed rapidly and easily in plants.

EXAMPLE - Immunogenic scFv protein designated CJ was derived from human lymphoma patient (having the initials CJ) and had as its linker (Gly4Ser)3. DNA fragments encoding the dual domain scFv fragments having the V regions of the CJ human lymphoma were generated and cloned into a modified TTO1A vector, containing a hybrid fusion of TMV and ToMV (Kumagai, M H et al., (1995) Proc. Natl. Acad.

Sci. USA. 92:1679-1683). In this vector, a tobacco mosaic virus (TMV) coat protein subgenomic promoter was located upstream of the insertion site of the CJ sequence. Following infection, this TMV coat protein subgenomic promoter directs initiation of the CJ RNA synthesis in plant cells at the transcription start point (tsp). The rice alpha amylase signal peptide, fused in-frame to the CJ sequence, encodes a 31 residue polypeptide which targets proteins to the secretory pathway and was subsequently cleaved off between the C- terminal Gly of the signal peptide and the N-terminal Met of the expressed CJ scFv protein. The sequence encoding CJ scFv was introduced between the 30K movement protein and the ToMV coat protein (Tcp) genes. An SP6 phage promoter was introduced upstream of the viral cDNA, allowing for transcription of infective genomic plus-strand RNA. Capped infectious RNA was made in vitro from 1 micro-g PmeI-linearized plasmid, using an SP6 message kit. Synthesis of the message was quantified by gel electrophoresis and 2 microg of the in vitro transcribed viral RNA was applied with an abrasive to the lower leaves (1-2 cm in size) of Nicotiana benthamiana. Transcription of subgenomic RNA encoding the CJ scFv protein was initiated after infection at the indicated transcription start point. High levels of subgenomic RNA species were synthesized in virus-infected plant cells and served as templates for the translation and subsequent accumulation of CJ scFv protein. Signs of infection were visible after 5-6 days as mild leaf deformation, with some variable leaf mottling and growth retardation. Eleven to fourteen days post inoculation, the secreted proteins were isolated. Leaf and stem material was harvested, weight and then subjected to a 700 mm Hg vacuum for 2 minutes in infiltration buffer (100 mM Tris HCl, pH 7.5 and 2 mM ethylenediaminetetraacetic acid (EDTA)). Secreted proteins termed interstitial fraction or IF) were recovered from infiltrated leaves by mild centrifugation concentrated. Total protein was measured. The secreted material was analyzed for the presence of soluble CJ scFv protein by the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS- PAGE) followed by Western blot with CJ mAb 7D11 specific for the idiotype of CJ. Western blots were developed by enhanced chemiluminescence (ECL). No cross reactivity to plant proteins was observed. Individual clones were sequenced, analyzed for reading frame and amino acid identity to the original CJ Ig sequence, and then screened for protein expression in infected plants. Clones 20 and 30 showed high levels of expression, as well is accumulation of protein dimers. Clone C contained a modification of the (Gly3Ser)4 linker. The quality of CJ protein, optimized by the random linker library, was validated by a functional assay in animals. Clone CJLL20 was purified by 7D11 affinity chromatography, administered to five mice in 3 bi-weekly immunizations of 30 micro-g each. Ten days after the third injection, serum was sampled. Using the native idiotype (ID12), or an isotype-matched irrelevant human antibody in a sandwich enzyme linked immunosorbent assay (ELISA), the sera were tested for specific responses to the CJ idiotype. The sera of all 5 mice had high titers of anti-CJ antibodies. Thus, the immune response induced by the vaccine was highly specific for variable heavy (VH) and variable light (VL) regions of the original Ig. These results suggested that the protein produced in plants was folded correctly so that it could induce an appropriate immune response in immunized subjects. (48 pages)

L4 ANSWER 10 OF 24 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2003-26799 BIOTECHDS
TI Binders based on dimerised immunoglobulin V-H domains; antibody against antigen production via phage display library
AU SEPULVEDA J; JIN HL; SBLATTERO D; BRADBURY A; BURRONE OR
CS Int Ctr Genet Engn and Biotechnol; Univ Trieste; Los Alamos Natl Lab
LO Burrone OR, Int Ctr Genet Engn and Biotechnol, Area Sci Pk,Padriciano 99, I-34012 Trieste, Italy
SO JOURNAL OF MOLECULAR BIOLOGY; (2003) 333, 2, 355-365 ISSN: 0022-2836

DT Journal
LA English
AN 2003-26799 BIOTECHDS
AB AUTHOR ABSTRACT - Antibody binding to antigen is mediated by the surface formed by the association of the two variable (V) regions of the L (V-L) and H (V-H) chains. The capacity Of V-L to dimerise and the high structural similarity Of V-L and V-H domains suggested the possibility that V-H could also associate. We show here that spontaneous formation of V-H dimers (VHD) is in many cases permissive, producing stable molecules with antigen binding specificity. VHD were displayed on filamentous phages for the selection of antigen-specific binders. VHD were expressed and secreted efficiently from both bacteria and mammalian cells in different formats, including single-chain (V-H(1)-linker-V-H(2)), double chain ((V-H)(2)) and IgG analogues having the V-L replaced by V-H. The affinity (K-d,K-app) achieved with a VH dimer expressed in the IgG format, specific for a glutenin subunit was around 30 nM measured by two different methods, which was about 20 times higher than that corresponding to the V-L/V-H counterpart. (C) 2003 Elsevier Ltd. All rights reserved.
DERWENT ABSTRACT: Phages from individual colonies were used to infect HB2151 strain. Bacteria were grown in 2 X YT ampicilin medium at 37 deg to 0.5 A600 nm induced with 0.5 mM IPTG and incubated at 30 deg overnight. The periplasmic single chain antibody (scFv) fraction was prepared after four hours of induction by osmotic shock in PPB buffer (200 mg/ml of sucrose, mM EDTA, 30 mM Tris-HCl pH 8), for 20 minutes at 4 deg, centrifuged and the pellet washed with 5 mM MgSO4. Pooled supernatants were dialysed against phosphate buffered saline, PBS. The scFv were purified using the Ni-NTA ki(11 pages)

L4 ANSWER 11 OF 24 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2003-02959 BIOTECHDS
TI Modified single chain multimeric Fv antibody acting as a signal transduction agonist for treatment of inflammatory hormonal and blood disorders and cancer;
vector-mediated recombinant monoclonal antibody gene transfer and expression in host cell for use in diabetes, autoimmune disease, leukemia and blood disorder prevention and therapy
AU FUKUSHIMA N; TSUCHIYA M; UNO S; OHTOMO T; YABUTA N; TSUNODA H
PA CHUGAI SEIYAKU KK
PI WO 2002033073 25 Apr 2002
AI WO 2001-JP9260 22 Oct 2001
PRAI JP 2001-277314 12 Sep 2001; JP 2000-321821 20 Oct 2000
DT Patent
LA Japanese
OS WPI: 2002-682599 [73]
AN 2003-02959 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A new modified single chain Fv antibody (I) contains at least two H chain V domains and at least two L chain V domains from the same or different monoclonal antibodies and which is an agonist for crosslinking a molecule at the cell surface or within the cell and thereby transducing a signal into the cell.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) DNA encoding the antibody (I); (2) animal cells and microorganisms transformed by and expressing the DNA of (1); (3) the preparation of (I) by culture of the transformed cells; (4) drug compositions containing (I); (5) an assay method for the agonist activity of (I) by contact of (I) with cells expressing the relevant cell surface or internal molecule.
BIOTECHNOLOGY - Preferred Antibody: The H and L chain V domains are joined by a peptide linker sequence. The single chain Fv antibody (scFv) is multimeric, preferably a tetramer, trimer or dimer, and may be humanised or of fully human origin. Preferred

Molecule: This is a hormone receptor, cytokine receptor, tyrosine kinase receptor or intranuclear receptor, for example a receptor for erythropoietin (EPO), TPO, granulocyte-colony stimulating factor (G-CSF), macrophage-colony stimulating factor (M-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), tumor necrosis factor (TNF), interleukins, interferons, growth hormone, insulin, stem cell factor (SCF), vascular endothelial growth factor (VEGF), endothelial growth factor (EGF), nerve growth factor (NGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), tumor growth factor beta (TGF-beta), LIF, CNTF, OSM, Notch family, E2F, E2F/DP1 or TAK1/TAB1.

ACTIVITY - Cytostatic; Antiinflammatory; Immunosuppressive; Immunostimulant. No biological data given.

MECHANISM OF ACTION - Agonist for cell signal transduction; Apoptosis induction; Cell proliferation induction; Cell differentiation induction; Cell cycle regulation; Cell lysis induction; Cell adhesion inhibition. No biological data given.

USE - Treatment and prevention of cancer, inflammatory disease, hormonal disorders including diabetes, autoimmune disease, leukemia, and blood disorders.

EXAMPLE - Plasmid vector pscM2DEm02 (see drawing) is constructed containing DNA encoding the H chain and L chain V domains of a humanised mouse anti-human IAP monoclonal antibody (MABL-2) separated by DNA encoding a GGGGSGGGGSGGGGS linker peptide. This is used to transform Escherichia coli BL21(DE3)pLysS. The scFv antibody is isolated from the culture by ultrasound disruption of the cells , then purification of Sephacryl S-300 and Superdex 200pg (Amersham). Both monomer and dimer scFv are obtained. SCID mice are injected subcutaneously with KPMM2 cells (JP07236475) (6 x 106 cells/mouse). During the three days following the challenge the mice are given three doses of 0.1 mg of the antibody. After this time the level of human IgG in the blood of the mice is measured by ELISA assay. This level is about 1 mg/ml after administration of dimeric antibody and 7 mg/ml using monomeric antibody; mice challenged with KPMM2 cells but not treated with antibody have a human IgG level of about 8.5 mg/ml. The dimeric antibody is thus strongly inhibitory of KPMM2 cell proliferation. (218 pages)

L4 ANSWER 12 OF 24 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2002-17629 BIOTECHDS
TI A targeting molecule for use in forming complexes to treat cancer, such as adenocarcinoma of the prostate, comprises a soluble adenoviral receptor domain, a trimerization domain and a targeting ligand domain; adeno virus receptor gene transfer and expression in COS cell , useful for gene therapy
AU KIM J G; SMITH T; STEVENSON S C; KALEKO M
PA NOVARTIS AG; NOVARTIS-ERFINDUNGEN VERW GES MBH
PI WO 2002029072 11 Apr 2002
AI WO 2000-EP11514 6 Oct 2000
PRAI US 2000-684552 6 Oct 2000
DT Patent
LA English
OS WPI: 2002-471317 [50]
AN 2002-17629 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A targeting molecule (I) comprises a soluble adenoviral receptor domain, a trimerization domain and a targeting ligand domain, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a trimeric targeting molecule comprising (I); (2) a complex comprising an adenoviral particle and (I); (3) a polynucleotide encoding (I); (4) an expression vector comprising the polynucleotide; (5) targeting (M1) an adenoviral particle to a cell which expresses

a cell surface molecule comprising; (a) contacting an adenoviral particle with (I) to form a complex comprising the adenoviral particle and (I); and (b) contacting the cell with the complex. (6) delivering (M2) a heterologous gene selectively to a cell which expresses a cell surface molecule comprises: (a) contacting an adenoviral particle which comprises the heterologous gene with (I) to form a complex suitable to target a cell surface molecule; and (b) contacting cell with complex; (7) identifying (M3) either or both, a cell surface molecule that is suitable for mediating cell entry of an adenoviral particle to a specific cell or tissue expressing cell surface molecule, or a ligand suitable for targeting an adenoviral particle to a specific cell or tissue , comprises: (a) combining a ligand molecule for a cell surface molecule with a soluble adenoviral receptor molecule and a trimerization domain to form a targeting molecule; (b) contacting an adenoviral particle which comprises a marker gene with the targeting molecule to form a complex; (c) contacting a cell or tissue expressing the cell surface molecule with the complex; and (d) selecting a complex able to transduce cell or tissue as reported by the reporter gene; and (8) treatment of a disease with an adenoviral gene therapy comprises contacting a trimeric targeting molecule with an adenoviral gene therapy vector to form a complex, and administering the complex therapeutically.

WIDER DISCLOSURE - Also disclosed are the following: (1) (I) useful to assess whether a cell surface molecule is capable of mediating cell entry of the adenoviral particle; and (2) screening targeting ligand domains in the cell culture.

BIOTECHNOLOGY - Preferred Molecule: The soluble adenoviral receptor domain in (I) is sCAR (soluble coxsackie adenovirus receptor). The trimerization domain is derived from a leucine zipper molecule, the isoleucine variant of the yeast GCN4 leucine zipper molecule, fused with the soluble adenoviral receptor domain or fused at the carboxy-terminal end of the soluble adenoviral receptor domain. (I) also comprises a linker element which is localized between the carboxy-terminal end of the soluble adenoviral receptor domain and the trimerization domain. The linker element can consist of alternating glycine and serine residues. The targeting ligand domain is a cyclic RGD, includes at least 15 amino acids derived from an apoE protein, includes two tandem copies of amino acids 141-155 derived from apoE protein or is conjugated to the carboxy-terminus of the soluble adenoviral receptor doamin. (I) further comprises a linker element which is localized between the carboxy-terminal end of the trimerization domain and the targeting ligand doamin. The soluble adenoviral receptor domain is sCAR and the trimerization domain is derived from a leucine zipper molecule. The targeting ligand domain comprises a single chain antibody (scFv). Preferred Complex: The soluble adenoviral receptor domain is sCAR. The trimerization domain is derived from a leucine zipper molecule. The complex further comprises a linker element which is localized between the carboxy-terminal end of the soluble adenoviral receptor domain and the trimerization domain. The adenoviral receptor domain is sCAr and the trimerization domain is derived from a leucine zipper molecule. The adenoviral particle further comprises a heterologous gene and is an oncolytic adenoviral particle. Preferred Method: In (M1), the adenoviral particle is an oncolytic adenoviral particle. In (M4), the disease is cancer, perferably an adenocarcinoma of the prostate. The side effects of gene therapy and adenoviral liver toxicity is reduced. Preparation: (I) is prepared by standard genetic recombinant techniques.

ACTIVITY - Cytostatic; hepatotropic; virucide. No supporting data is given.

MECHANISM OF ACTION - Gene therapy. No supporting data is given.

USE - (I) is used for targeting an adenoviral particle to a cell expressing a cell surface molecule, comprising contacting the adenoviral particle with (I) to form a complex, and

contacting the cell with the complex, and in delivering a heterologous gene selectively to a cell. The complex is used for preparing a medicament for treatment of disease in a human mammal, such as cancer, preferably, adenocarcinoma of the prostate, by gene therapy (all claimed).

ADMINISTRATION - No administration or dosage details are given.

ADVANTAGE - (I) consists of a conjugate which binds to the fiber knob of the adenoviral particle to form a complex and redirects the vector to a different cell surface molecule. The trimerization of the soluble adenoviral receptor domain significantly enhances the binding of (I) to the adenoviral particle and can more efficiently transduce cells in vitro and in vivo. The re-targeting of an adenoviral particle does not require the time-consuming generation of genetically modified adenoviral vectors, and adenoviral vectors can be prepared and grown to high titer.

EXAMPLE - 35S-labeled AD5 fiber protein was mixed with purified sCAR protein and subjected to electrophoresis on a non-denaturing acrylamide gel. Autoradiography of the dried gel demonstrated that the fiber was bound to the trimeric form of sCAR. Bands were not visible at positions corresponding to monomeric or dimeric forms of sCAR. However, comassie staining of non-denaturing gels showed that the large majority of sCAR was i monomeric form. This suggests that the trimers of sCAR had a significantly higher affinity for fiber than either monomers or dimers. To increase the abundance of trimers of sCAr,a sequence encoduing the GCN4 trimerization domain was inserted into the sCAR expression plasmid at the 3' end of the sequence encoding the extracellular domain of CAR. T o increase the likelihood that the trimerization domains would be accessible to one another to promote the formation of sCAr trimers,a linker consisting of alternating glycine and serine residues was inserted between the end of sCAR and the trimerization domain. The resulting expression plasmid was introduced into COS cells by electroporation and the sCAR protein was isolated and purified.(75 pages)

L4 ANSWER 13 OF 24 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2002-13922 BIOTECHDS
TI Immunoconjugate compositions for treating cancer by inhibiting angiogenesis and for delivering a diagnostic agent to tumor, comprises anti-vascular endothelial growth factor antibody attached to a biological agent;
monoclonal antibody preparation by hybridoma cell culture and mouse spleenocyte, myeloma cell fusion for disease diagnosis or therapy
AU THORPE P E; BREKKEN R A
PA UNIV TEXAS SYSTEM
PI AU 200179401 6 Dec 2001
AI AU 2000-79401 28 Apr 2000
PRAI AU 2001-79401 12 Oct 2001
DT Patent
LA Unavailable AU
OS WPI: 2002-281368 [33]
AN 2002-13922 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A composition (I) comprises an immunoconjugate comprising an anti-vascular endothelial growth factor (VEGF) antibody (Ab) (or its antigen-binding fragment), attached to a biological agent, where the Ab binds to same epitope as monoclonal antibody (MAb) 2C3 ATCC PTA 1595, is new.

DETAILED DESCRIPTION - A composition (I) comprises an immunoconjugate comprising an anti-vascular endothelial growth factor (VEGF) antibody (Ab) (or its antigen-binding fragment), attached to a biological agent, where the Ab binds to same epitope as monoclonal antibody (MAb) 2C3 ATCC PTA 1595 and significantly inhibits VEGF binding to VEGF receptor VEGFR2 (KDR/Flk-1) without inhibiting VEGF binding to

VEGF receptor VEGFR1 (Flt-1). INDEPENDENT CLAIMS are also included for the following: (1) a kit comprising (I); (2) an immunoconjugate comprising MAb ATCC PTA 1595 operatively attached to a first biological agent; and (3) preparing (II) an immunoconjugate that comprises anti-VEGF Ab that binds to the same epitopes as the MAb ATCC PTA 1595.

BIOTECHNOLOGY - Preparation: (II) involves immunizing a non-human animal, preferably a transgenic mouse that comprises a human Ab library with composition comprising a first immunogenic VEGF component, selecting from the immunized animal an Ab that cross-reacts with MAb ATCC PTA 1595, and operatively attaching the selected Ab to a first biological agent. The method comprises obtaining the nucleic acids that encode the anti-VEGF Ab and expressing the nucleic acids to obtain a recombinant anti-VEGF Ab. (II) involves administering to a non-human animal, a first immunogenic VEGF component, preparing a combinatorial immunoglobulin phagemid library expressing RNA isolated from the spleen of the immunized animal, selecting from the phagemid library a clone that expresses an anti-VEGF Ab that cross-reacts with the monoclonal Ab 2C3 (ATCC PTA 1595), expressing the anti-VEGF Ab-encoding nucleic acids from the selected clone to provide a recombinant anti-VEGF Ab, and operatively attaching the recombinant anti-VEGF Ab to a first biological agent (all claimed). Preferred Antibody: The anti-VEGF Ab is a monoclonal, human, humanized or part-human Ab or its antigen-binding fragment, IgG or IgM Ab, scFv, Fv, Fab', Fab, linear Ab or F(ab')2 antigen-binding fragment of an Ab, or chimeric or recombinant Ab. Ab is a dimer , trimer or multimer of the Ab. The Ab comprises an antigen-binding region of the antibody operatively attached to a human antibody framework or constant region. Ab comprises a first variable region that includes a sequence of 127 or 115 amino acids fully defined in the specification. Ab is operatively attached to alkaline phosphatase, that cleaves an inactive phosphate-prodrug, to release an active drug. Ab is operatively attached to a therapeutic or diagnostic agent, including chemotherapeutic agent; radiotherapeutic agent; anti-angiogenic agent; anti-tubulin drug; a coagulant; tissue factor; apoptosis-inducing agent; steroid; antimetabolite; anthracycline; vinca alkaloid; antibiotic; cytokine; alkylating agent; or coagulating agent. The anti-angiogenic agent is angiopoietin-2, angiopoietin-1, angiostatin, vasculostatin, canstatin, maspin, or endostatin. The anti-tubulin drug is colchicine, taxol, vinblastine, vincristine, vindescine, or combretastin. The coagulating agent is Factor II/IIa, Factor VII/VIIa, FactorIX/IXa, Factor X/Xa, a vitamin K-dependent coagulation factor that lacks the Gla modification, Russell's viper venom Factor X activator, thromboxane A2, thromboxane A2 synthase or alpha2-antiplasmin. It is also a human tissue factor, a mutant tissue factor deficient in the ability to activate Factor VII, truncated tissue factor, or dimeric, trimeric or polymeric tissue factor, or tissue factor derivative. The diagnostic agent is an imaging or detectable agent; an X-ray detectable compound; a radioactive ion or a nuclear magnetic spin-resonance isotope; biotin, avidin or to an enzyme that generates a colored product upon contact with a chromogenic substrate. Ab is operatively attached to a cytotoxic, cytostatic or anticellular agent capable of killing or suppressing the growth or cell division of endothelial cells; or is attached to a plant-, fungus- or bacteria-derived toxin. The toxin is A chain toxin, a ribosome inactivating protein, alpha-sarcin, gelonin, aspergillin, testrictocin, a ribonuclease, an epipodophyllotoxin, diphtheria toxin, or Pseudomonas exotoxin, preferably a ricin A chain or deglycosylated ricin A chain. Ab is attached to the biological agent as a fusion protein prepared by expressing a recombinant vector that comprises, in the same reading frame, a DNA segment encoding the Ab operatively linked to a DNA segment encoding the biological agent. Ab is attached to the agent directly or by a biologically releasable bond or selectively cleavable linker, preferably a peptide linker that includes a cleavage site for urokinase, pro-urokinase, plasmin, plasminogen, tumor growth factor beta, staphylokinase, thrombin, Factor IXa, Factor Xa, a

mettaloproteinase, an interstitial collegenase, a gelatinase or a stromelysin. Ab is attached a second Ab, or its antigen binding region, that binds to the therapeutic or diagnostic agent. (I) is a liposomal formulation, and further comprises a second therapeutic agent. The second therapeutic agent is an anti-cancer agent is selected from a chemotherapeutic agent, a radiotherapeutic agent, anti-angiogenic agent, apoptosis inducing agent, anti-tubulin drug or their tumor targeted agents. The agent is angiopoietin-1, or endostatin. Alternatively, the anti-cancer agent is an antibody-therapeutic agent construct comprising a therapeutic agent that is operatively linked to a second antibody, or its antigen binding fragment, that binds to a surface-expressed, surface -accessible or surface-localized component of a tumor cell, tumor vasculature or tumor stroma.

ACTIVITY - Ophthalmological; Antiseborrheic; Antirheumatic; Dermatological; Antiinflammatory; Anti-HIV; Antiulcer; Vasotropic; Vulnerary; Virucide; Cytostatic; Antibacterial; Protozoacide; Osteopathic; Antiarthritic; Antianemic; Antidiabetic; Tranquilizer; Antipsoriatic; Antiatherosclerotic; Gynecological; Antithyroid; Fungicide; Antisickling.The anti-tumor effect of 2C3 was evaluated. Male nu/nu mice were injected subcutaneously with 1 x 10 (to the power of 7) NCI-H358 non-small cell lung cancer (NSCLC) cells or 5 x 10 (to the power of 6) A673 rhabdomyosarcoma cells. Mice bearing subcutaneous NCI-H358 tumors or A673 200-400 mm3 in size were injected intraperitoneally (i.p.) with test or control antibodies. The NCI-H358 bearing mice were treated with 100 micrograms of antibodies per injection three times a week during the first week and twice a week during the second and third week. The mice were then switched to 50 micrograms/injection every 5 days. Mice were sacrificed when their tumors reached 2500 mm3 in size. The results showed that 2C3 inhibited the in vivo growth of both NCI-H358 NSCLC and A673 rhabdomyosarcoma in nu/nu mice in a dose dependent manner. One hundred micrograms 2C3 given i.p. 2 times per week to mice that was injected with tumor cells subcutaneously one day earlier inhibited the growth of both human tumor types. The final tumor volume in the 2C3 recipients was 150 mm3 in both tumor systems, as compared with 1000 mm3 in the recipients of controls.

MECHANISM OF ACTION - Inhibitor of angiogenesis and inducer of tumor regression; VEGF binding to VEGFR2 inhibitor; VEGF-induced endothelial cell proliferation inhibitor; VEGF induced vascular permeablility inhibitor.

USE - (I) is useful in therapy, diagnosis, for inhibiting angiogenesis in an animal having ocular neovascular disease or macular degeneration, for delivering a biological agent (diagnostic or therapeutic) to a vascularized tumor, in the manufacture of a medicament for treating a disease by inhibiting angiogenesis, in therapy without substantial inhibiting of macrophages, osteoclasts or chondroclasts and for treating cancer in an animal having or is at risk for developing, a vascularized solid tumor, a metastatic tumor or metastases from a primary tumor. (I) is useful in detecting VEGF, inhibiting VEGF binding to the VEGF receptor VEGFR2, without significantly inhibiting VEGF binding to VEGFR1, for specifically inhibiting VEGF-induced endothelial cell proliferation, without significantly inhibiting VEGF-induced macrophage, osteoclast or chondroclast function, and for treating an angiogenic disease. Cancer can be treated by administering (I) to a mammal that has vascularized solid tumor and subsequently administering a composition comprising an inactive pro-drug that is cleaved by biological agent attached to the Ab in (I), to release the drug specifically within the tumor vasculature or stroma (all claimed). (I) is useful for treating arthritis, rheumatoid arthritis, psoriasis, atherosclerosis, diabetic retinopathy, Grave's disease, restenosis, inflammatory diseases and disorders, acne rosacea, acquired immune deficiency syndrome, atopic keratitis, bacterial ulcers, Bechet's disease, blood borne tumors, carotid obstructive disease, chemical burns, chronic inflammation, chronic retinal detachment, chronic uveitis, corneal graft rejection, Crohn's disease, epidemic keratoconjunctivitis,

fungal ulcers, Herpes simplex infections, Kaposi's sarcoma, Mycobacterial infections other than leprosy, ocular neovascular disease, myopia, osteoarthritis, Paget's disease, protozoan infections, retinal neovascularization, scleritis, sickle cell anemia, Sogren's syndrome, systemic lupus, trauma, ulcerative colitis, vitamin A deficiency, Wegeners sarcoidosis, and endometriosis. Other diseases that can be treated include diseases such as diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue.

ADMINISTRATION - (I) is formulated for parenteral administration (claimed), intravenous, intramuscular, subcutaneous or transdermal route. Dosage is 10-100 mg/m2, preferably 35-90 mg/m2.

ADVANTAGE - Anti-VEGF antibody is highly specific and has improved safety due to its specific blocking properties.

EXAMPLE - Peptides corresponding to the N-terminal 26 amino acids of human vascular endothelial growth factor (huVEGF) and the N-terminal 25 amino acids of guinea pig VEGF (gp VEGF) were synthesized and conjugated by the C-terminal cysteine to thyroglobulin. For the production of anti-gp VEGF antibody producing hybridomas, C57/Bl-6 mice were immunized with the gpVEGF-peptide-thyroglobulin conjugate. For the production of anti-human VEGF antibodies, BALB/c mice were immunized with either the huVEGF-peptide-thyroglobulin conjugate or recombinant human VEGF. 3 days after the final boost spleenocytes were fused with myeloma P3X63AG8.653 and were cultured as described by Morrow et al., Colloidal Gold: Principles, Methods and Applications, pp. 31-57, (1990). IgG antibodies (2c3, 12D7, 3E7) were purified from tissue culture supernatant. There were marked differences in the ability of the antibodies to bind to soluble VEGF in free and complexed form. 2C3 and 12D7 displayed a preference for free VEGF, with half-maximal binding being attained at 1 and 20 nM respectively as compared with 150 and 250 nM respectively for the VEGF receptor VEGFR2 (VEGF:Flk-1) complex. However, 2C3 localized to tumor vasculature, as well as tumor stroma, after infection in vivo. 3E7 bound equally well to free VEGF and the VEGF:Flk-1 complex, with half-maximal binding being attained at 1 nM for both.(299 pages)

L4 ANSWER 14 OF 24 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
AN 2001:32662527 BIOTECHNO
TI Construction, expression and characterisation of a single-chain diabody derived from a humanised anti-Lewis Y cancer targeting antibody using a heat-inducible bacterial secretion vector
AU Power B.E.; Caine J.M.; Burns J.E.; Shapira D.R.; Hattarki M.K.; Tahtis K.; Lee F.-T.; Smyth F.E.; Scott A.M.; Kortt A.A.; Hudson P.J.
CS B.E. Power, CSIRO Health Sciences and Nutrition, 343 Royal Parade, Parkville, Vic. 3052, Australia.
E-mail: barbara.power@hsn.csiro.au
SO Cancer Immunology, Immunotherapy, (2001), 50/5 (241-250), 34 reference(s)
CODEN: CIIMDN ISSN: 0340-7004
DT Journal; Article
CY Germany, Federal Republic of
LA English
SL English
AB A single-chain antibody fragment (scFv) of the humanised monoclonal antibody, hu3S193, that reacts specifically with Le.sup.y antigen expressed in numerous human epithelial carcinomas was constructed. A five-residue linker joined the C-terminus of the V.sub.H and the N-terminus of the V.sub.L, which prevented V-domain association into a monomeric scFv and instead directed non-covalent association of two scFvs into a dimer or diabody. The diabody was secreted into the E. coli periplasm using a heat-inducible vector, pPOW3, and recovered as a soluble, correctly processed protein, following osmotic shock or solubilised with 4M urea from the insoluble fraction. The diabody from both fractions was isolated by a rapid batch affinity chromatography procedure, using the FLAG affinity tag to minimise degradation and aggregation. The purified

diabody has an M.sub.r of .apprx.54 kDa, was stable and demonstrated similar binding activity as the parent monoclonal antibody, as measured by FACS and BIAcore analyses. The radio-labelled diabody showed a rapid tumour uptake, with fast blood clearance, proving it to be an excellent potential candidate as a tumour-imaging agent.

L4 ANSWER 15 OF 24 CAPLUS COPYRIGHT 2006 ACS on STN
AN 2000:646167 CAPLUS
DN 133:234223
TI Target cell-specific multivalent proteins and their use in transformation and therapy
IN Kontermann, Roland; Nettelbeck, Dirk; Sedlacek, Hans-Harald; Muller, Rolf
PA Aventis Pharma Deutschland G.m.b.H., Germany
SO PCT Int. Appl., 81 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000053790	A1	20000914	WO 2000-EP1612	20000226
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
DE 19910419	A1	20000921	DE 1999-19910419	19990310
EP 1161550	A1	20011212	EP 2000-910717	20000226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002537847	T2	20021112	JP 2000-603411	20000226
PRAI DE 1999-19910419	A	19990310		
WO 2000-EP1612	W	20000226		

AB The invention relates to a target cell-specific, multivalent protein (MVP) characterized in being comprised of the following components which are covalently bound to one another: (a) a binding structure (a)n specific for the vector; (b) a linker (b)m and; (d) at least two binding structures (c)o for the target cell, whereby, independent of one another, n=1-10, m=1-10, and o=2-10. The invention also relates to the production and use of said MVP. Thus, a protein comprising an anti-adenovirus fiber protein single-chain Fv linked, via a linker peptide, to VEGF2 was prepared with recombinant cells . This protein dimerizes upon formation of disulfide bonds between the VEGF2 moieties. The dimer protein may be used to enhance transduction with adenoviral vectors.
RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 16 OF 24 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
AN 2000:30802056 BIOTECHNO
TI Designer genes: Recombinant antibody fragments for biological imaging
AU Wu A.M.; Yazaki P.J.
CS A.M. Wu, Department of Molecular Biology, Beckman Res. Inst. of City of Hope, 1450 East Duarte Road, Duarte, CA 91010, United States.
E-mail: awu@coh.org
SO Quarterly Journal of Nuclear Medicine, (2000), 44/3 (268-283), 93 reference(s)
CODEN: QJNMF7 ISSN: 1124-3937
DT Journal; General Review
CY Italy

LA English
SL English
AB Monoclonal antibodies (MAbs), with high specificy and high affinity for their target antigens, can be utilized for delivery of agents such as radionuclides, enzymes, drugs, or toxins in vivo. However, the implementation of radiolabeled antibodies as 'magic bullets' for detection and treatment of diseases such as cancer has required addressing several shortcomings of murine MAbs. These include their immunogenicity, sub-optimal targeting and pharmacokinetic properties, and practical issues of production and radiolabeling. Genetic engineering provides a powerful approach for redesigning antibodies for use in oncologic applications in vivo. Recombinant fragments have been produced that retain high affinity for target antigens, and display a combination of rapid, high-level tumor targeting with concomitant clearance from normal tissues and the circulation in animal models. An important first step was cloning and engineering of antibody heavy and light chain variable domains into single-chain Fvs (molecular weight, 25-27 kDa), in which the variable regions are joined via a synthetic linker peptide sequence. Although scFvs themselves showed limited tumor uptake in preclinical and clinical studies, they provide a useful building block for intermediate-sized recombinant fragments. Covalently linked dimers or non-covalent dimers of scFvs (also known as diabodies) show improved targeting and clearance properties due to their higher molecular weight (55 kDa) and increased avidity. Further gains can be made by generation of larger recombinant fragments, such as the minibody, an scFv -C(H)3 fusion protein that self-assembles into a bivalent dimer of 80 kDa. A systematic evaluation of scFv, diabody, minibody, and intact antibody (based on comparison of tumor uptakes, tumor:blood activity ratios, and calculation of an Imaging Figure of Merit) can form the basis for selection of combinations of recombinant fragments and radionuclides for imaging applications. Ease of engineering and expression, combined with novel specificities that will arise from advances in genomic and combinatorial approaches to target discovery, will usher in a new era of recombinant antibodies for biological imaging.

L4 ANSWER 17 OF 24 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
AN 1999:29428939 BIOTECHNO
TI Pharmacokinetics and biodistribution of engineered single-chain antibody constructs of MAb CC49 in colon carcinoma xenografts
AU Pavlinkova G.; Beresford G.W.; Booth B.J.M.; Batra S.K.; Colcher D.
CS Dr. D. Colcher, Coulter Pharmaceutical Inc., 600 Gateway Blvd., South San Francisco, CA 94080, United States.
SO Journal of Nuclear Medicine, (1999), 40/9 (1536-1546), 26 reference(s) CODEN: JNMEAQ ISSN: 0161-5505
DT Journal; Article
CY United States
LA English
SL English
AB Monoclonal antibodies (MAbs) have been proven useful in clinical studies for both diagnostic and therapeutic applications. The single-chain Fv (scFv) construct made from MAbs has potential applications for improved cancer diagnosis and therapy. A new CC49 scFv construct recognizing a tumor- associated mucin, TAG-72, was engineered and evaluated by immunological, pharmacokinetic and biodistribution analysis. Methods: The CC49 scFv construct was generated in which the VL and VH variable region genes were joined together with a 25-amino acid helical linker (205C). The new CC49 scFv (205C) was expressed as a monomer as well as a stable noncovalent dimer (¢scFv!.sub.2). The pharmacokinetic, biodistribution and tumor targeting characteristics of radiolabeled CC49 scFv were compared with CC49 IgG and enzymatically derived fragments F(ab').sub.2 and Fab', using the athymic mice bearing human colon cancer xenografts. Results: The association constant (K(A0) for the

intact CC49, dimeric scFv (scFv).sub.2 and monomeric scFv were 1.7 x 10.sup.9, 1.99 x 10.sup.9 and 0.52 x 1069 M-1 by Scatchard analysis and 1.14 x 10.sup.8, 4.46 x 10.sup.7 and 1.5 x 10.sup.7 M.sup.-.sup.1, respectively, by BIAcore analysis. Pharmacokinetic studies showed that more than 50% of monomeric scFv (.sim.27 kDa) was cleared from the blood in less than 10 min. The CC49 Fab' generated enzymatically from the parent murine Mab' (50 kDa) had a blood clearance that was faster than that of the (scFv).sub.2 (60 kDa) with half of the activity cleared from the serum within 30 and 50 min, respectively. The CC49 dimeric scFv (205C) showed a two-fold higher tumor uptake (than scFv or Fab') reaching 10 %ID/g at 60 min after injection. The scFv dimer also showed an excellent stability and increased avidity in vivo compared with the monomer, as demonstrated by the longer retention in tumor with 3 %ID/g remaining at 48 h. Conclusion: The rapid clearance from the blood, higher tumor uptake and longer retention of the stable dimer of CC49 scFv make it an important agent for potential imaging and therapeutic applications.

L4 ANSWER 18 OF 24 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN
AN 1999:29349135 BIOTECHNO
TI Single-chain antibodies in pancreatic cancer
AU Colcher D.; Pavlinkova G.; Beresford G.; Booth B.J.M.; Batra S.K.
CS D. Colcher, Department of Pathology Microbiology, University Nebraska Medical Center, 983135 Nebraska Medical Center, Omaha, NE 68198-3135, United States.
E-mail: dcolcher@unmc.edu
SO Annals of the New York Academy of Sciences, (1999), 880/- (263-280), 56 reference(s)
CODEN: ANYAA0 ISSN: 0077-8923
DT Journal; Conference Article
CY United States
LA English
SL English
AB Pancreatic cancer is a therapeutic challenge for surgical and medical oncology. Development of specific molecular tracers for the diagnosis and treatment of this lethal cancer has been one of our major goals. Monoclonal antibodies (MAbs) have been successfully used as selective carriers for delivering radionuclides, toxins or cytotoxic drugs to malignant cell populations; therefore, monoclonal antibody technology has led to a significant amount of research into optimizing targeted therapy. This targeted therapy results in the selective concentration of cytotoxic agents or radionuclides in tumors and should lessen the toxicity to normal tissues, which would normally limit the dosage and effectiveness of systemically administered drugs. The MAb CC49 reacts with a unique disaccharide, Sialyl-Tn, present on tumor-associated mucin (TAG-72) expressed by a majority of human adenocarcinomas. The unique Sialyl-Tn epitope has provided a potential target for immunotherapy of cancer. A single chain Fv (scFv) recombinant protein from CC49 MAb was prepared by engineering the DNA fragments for coding heavy-chain and light-chain variable regions with an appropriate oligonucleotide linker. scFv molecules, when compared to intact MAbs and the more conventional enzymatically derived F(ab').sub.2 and Fab' fragments, offer several advantages as carriers for the selective delivery of radionuclides to tumors. The divalent antibody fragments (sc(Fv).sub.2 or (scFv).sub.2) display an affinity constant similar to that of the intact CC49 IgG and are stable with storage, and after radiolabeling. In preclinical studies, both the covalent and the non-covalent dimeric scFvs exhibit excellent tumor targeting properties with characteristics similar to those of the monomer, e.g., the rapid blood clearance, low kidney uptake and small size suitable for rapid penetration through tumor tissue. Increased tumor targeting of the dimers are probably due to their increased functional affinity attributable to valency, coupled with their higher

molecular weight and fewer interactions with normal organs. These properties make these constructs superior to monovalent CC49 scFv . The relatively high tumor uptake, the in vitro and in vivo targeting specificity, and the stability in storage demonstrated by the dimeric CC49 sc(Fv).sub.2 makes it a promising delivery vehicle for therapeutic applications in pancreatic cancer.

L4 ANSWER 19 OF 24 Elsevier BIOBASE COPYRIGHT 2006 Elsevier Science B.V. on STN
AN 1999098745 ESBIOBASE
TI Production and characterization of an anti-(MUC1 mucin) recombinant diabody
AU Denton G.; Brady K.; Lo B.K.C.; Murray A.; Graves C.R.L.; Hughes O.D.M.; Tendler S.J.B.; Laughton C.A.; Price M.R.
CS G. Denton, Cancer Research Laboratories, School of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom.
SO Cancer Immunology Immunotherapy, (1999), 48/1 (29-38), 31 reference(s) CODEN: CIIMDN ISSN: 0340-7004
DT Journal; Article
CY Germany, Federal Republic of
LA English
SL English
AB A recombinant diabody fragment based on the anti-MUC1 monoclonal antibody, C595 has been produced in a bacterial expression system. Substitution of a 7-amino-acid linker sequence (Gly.sub.6Ser) for the original single-chain (sc)Fv 15-amino-acid linker (Gly.sub.4.sub.-Ser).sub.3, using polymerase- chain-reaction-based strategies, forces variable heavy (V(H)) and light (V(L)) domains to pair with complementary domains on neighbouring scFv molecules, forming a scFv dimer (diabody). This recombinant protein shows similar binding characteristics to the parental C595 monoclonal antibody. The ability to bind to MUC1 mucin on carcinoma cell surfaces will allow its potential as a diagnostic and therapeutic reagent of clinical utility to be investigated.

L4 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
AN 1998:806289 CAPLUS
DN 130:208617
TI IgM secretory tailpiece drives multimerization of bivalent scFv fragments in eukaryotic cells
AU Olafsen, Tove; Rasmussen, Ingunn B.; Norderhaug, Lars; Bruland, Oyvind S.; Sandlie, Inger
CS Division of Molecular Cell Biology, Department of Biology, University of Oslo, Oslo, N-0316, Norway
SO Immunotechnology (1998), 4(2), 141-153 CODEN: IOTEER; ISSN: 1380-2933
PB Elsevier Science B.V.
DT Journal
LA English
AB The monoclonal antibody (mAb) TP-3 binds selectively to human and canine osteosarcoma (OS) cells and is therefore a potential candidate for use as a targeting agent in radioimmunoimaging and therapy of OS metastases. However, intact murine mAbs have several drawbacks such as large size, delayed blood clearance and high immunogenicity, all of which can be overcome by genetic engineering. Objectives here were to construct and express bivalent and multivalent TP-3 scFv fragments from the mammalian expression vector, pLNO. This vector has unique restriction sites for simple cassette cloning of any individual variable (V) and constant (C) genes and has previously been used for expression of intact chimeric TP-3 mAbs and Fab fragments. Furthermore, it is also suitable for expression of any modified V region, such as a scFv fragment, fused to any modified C region or to non-Ig protein sequences. Six different constructs were made; 3 scFv-CH3 fragments that

differed in the design of linker between the scFv fragment and the IgG CH3 domain. These constructs were also made with the IgM secretory tailpiece (μtp) attached to the C terminus. All constructs were secreted as bivalent antibody fragments with a mol. weight of about 100 kDa. A band corresponding to a dimer appeared in all the supernatants from TP-3 scFv-CH3 producing cells, whether μtp was present or not, whereas higher orders of multimers were not seen. However, pulse chase analyses of the cells revealed that a small fraction of higher order polymers was formed from genes including the fragment encoding μtp and that μtp conferred retention both to.

RE.CNT 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 21 OF 24 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 2
AN 1997:559985 CAPLUS
DN 127:258195
TI Mammalian cell expression of dimeric small immune proteins (SIP)
AU Li, Erqiu; Pedraza, Alicia; Bestagno, Marco; Mancardi, Sabrina; Sanchez, Roberto; Burrone, Oscar
CS International Centre for Genetic Engineering and Biotechnology, Area Science Park, Trieste, 34012, Italy
SO Protein Engineering (1997), 10(6), 731-736
CODEN: PRENE9; ISSN: 0269-2139
PB Oxford University Press
DT Journal
LA English
AB We have designed and expressed bivalent small immune proteins (SIP) based on scFv fragments connected through a short linker of four amino acids to the CH3 domain of the human Ig γ1 H-chain. Three different versions have been designed and expressed in mammalian cells. In one construct a cysteine residue was included in the last amino acid of the flexible 15-amino acid long linker connecting the VL and VH domains, thus creating a disulfide bond stabilized mol. A version with a shorter (five amino acids) VL/VH linker was also produced and shown to be efficiently assembled and secreted. All three SIPs form dimers retaining their antigenic specificity in Western blotting and having a comparable functional affinity (avidity) as determined by ELISA.
RE.CNT 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 22 OF 24 BIOTECHNO COPYRIGHT 2006 Elsevier Science B.V. on STN DUPLICATE
AN 1996:26199751 BIOTECHNO
TI Minibody: A novel engineered anti-carcinoembryonic antigen antibody fragment (single-chain Fv-C(H)3) which exhibits rapid, high-level targeting of xenografts
AU Hu S.-Z.; Shively L.; Raubitschek A.; Sherman M.; Williams L.E.; Wong J.Y.C.; Shively J.E.; Wu A.M.
CS Department of Molecular Biology, City of Hope Beckman Res. Institute, 1450 East Duarte Road,Duarte, CA 91010, United States.
SO Cancer Research, (1996), 56/13 (3055-3061)
CODEN: CNREA8 ISSN: 0008-5472
DT Journal; Article
CY United States
LA English
SL English
AB A novel engineered antibody fragment (V(L)-V(H)-C(H)3, or 'minibody') with bivalent binding to carcinoembryonic antigen (CEA) was produced by genetic fusion of a T84.66 (anti-CEA) single-chain antibody (scFv) to the human IgG1 C(H)3 domain. Two designs for the connecting peptide were evaluated. In the T84.66/212 LD minibody, a two-amino acid

linker (generated by fusion of restriction sites) was used to join V(H) and C(H)3. In the T84.66/212 Flex minibody, the human IgG1 hinge plus an additional 10 residues were used as the connecting peptide. Size exclusion chromatography of purified minibodies demonstrated that both proteins had assembled into M(r)80,000 dimers as expected. Furthermore, analysis by SDS-PAGE under nonreducing conditions was consistent with disulfide bond formation in the hinge of the T84.66 Flex minibody. Purified minibodies retained high affinity for CEA (K(A), 2 x 10.sup.9 M.sup.-.sup.1) and demonstrated bivalent binding to antigen. Tumor targeting properties were evaluated in vivo using athymic mice bearing LS174T human colon carcinoma xenografts. .sup.1.sup.2.sup.3I-labeled T84.66 minibodies demonstrated rapid, high tumor uptake, reaching 17% injected dose/gram (%ID/g) for the LD minibody and 33%ID/g for the Flex minibody at 6 h following injection. Radioiodinated minibody also cleared rapidly from the circulation, yielding high tumor:blood uptake ratios: 44.5 at 24 h for the LD minibody and 64.9 at 48 h for the Flex minibody. Rapid localization by the T84.66/212 Flex minibody allowed imaging of xenografts at 4 and 19 h after administration.

L4 ANSWER 23 OF 24 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 1995-02650 BIOTECHDS
TI Improved tumor targeting with chemically crosslinked recombinant antibody fragments;
Fab' and single chain antibody Fv fragment antibody engineering, and chemical crosslinking to obtain dimer and trimer constructs
AU King D J; Turner A; Farnsworth A P H; Adair J R; Owens R J; Pedley R B; Baldock D; Proudfoot K A; Lawson A D G; Beeley N R A; Millar K; Millican T A; Boyce B A; Antoniw P; Mountain A; Begent R H J; Shochat D; Yarranton G T
CS Celltech; Roy.Free-Hosp.Sch.Med.London; Am.Cyanamid
LO Celltech Ltd., 216 Bath Road, Slough, Berkshire SL1 4EN, UK.
SO Cancer Res.; (1994) 54, 23, 6176-85
CODEN: CNREA8 ISSN: 0008-5472
DT Journal
LA English
AN 1995-02650 BIOTECHDS
AB To facilitate crosslinking of Fab' fragments, a chimeric B72.3 Fab' fragment was expressed with a hinge sequence containing a single cysteine residue. A vector was constructed in which the 2nd hinge cysteine was replaced by an alanine residue, and the construct was expressed in CHO cL18 cells. B72.3 scFv was also produced with a similar hinge region peptide attached to the C-terminus to allow crosslinking. This construct was expressed in Escherichia coli. The resulting fragments were crosslinked with linkers containing 2 or 3 maleimide groups to produce dimeric and trimeric molecules with increased avidity for antigen. Crosslinkers were also designed to contain a 12-N-4 macrocycle capable of stable radiolabeling with 90Y. Biodistribution studies in the nude mouse LS174T xenograft model with scFv, di-scFv amd tri-scFv showed that these fragments cleared extremely rapidly from the circulation, whereas di-Fab and tri-Fab accumulated relatively high levels of activity at the tumor. 90Y-labeled tri-Fab did no accumulate in kidney or bone, resulting in an attractive antibody fragment for tumor therapy. (50 ref)

L4 ANSWER 24 OF 24 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 4
AN 1992:126478 CAPLUS
DN 116:126478
TI Characterization of a recombinant single-chain molecule comprising the variable domains of a monoclonal antibody specific for human fibrin fragment D-dimer
AU Laroche, Yves; Demeyer, Marc; Stassen, Jean Marie; Gansemans, Yannick; Demarsin, Eddy; Matthyssens, Gaston; Collen, Desire; Holvoet, Paul
CS Corvas Int. NV, Ghent, Belg.
SO Journal of Biological Chemistry (1991), 266(25), 16343-9

CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB A recombinant single-chain mol., scFv-K12G0, containing the variable domains of the monoclonal antibody MA-15C5, specific for fragment D-dimer of human cross-linked fibrin, was constructed and expressed in Spodoptera frugiperda, Sf9, insect cells. The Arg108 C-terminal amino acid of the variable domain of the light-chain of the antibody was connected through a synthetic Ala-Gly-Gln-GLy-Ser-Ser-Val peptide linker with the Gln1 N-terminal amino acid of the variable domain of its heavy chain. ScFv-K12G0 was secreted by the infected Sf9 cells at a rate of 10 μg/106 cells within 48 h, resulting in conditioned medium with a maximum concentration of 15 mg of scFv-K12G0/L. The mol., purified to homogeneity by ion exchange chromatog. and gel filtration, migrated as a single Mr band on reduced SDS-gel electrophoresis. It bound to immobilized fragment D-dimer with an affinity constant of 4.0 + 109 M-1 (2.0 + 1010 M-1 for intact MA-15C5). Clearing of scFv-K12G0 from the circulation in rabbits occurred with an initial half-life (t1/2α) of 10 min and a clearance of 5.1 mL min-1, as compared to 90 min and 210 mL min-1 for intact MA-15C5. Nephrectomy resulted in a prolongation of t1/2α to 110 min, suggesting that the rapid clearance of scFv-K12G0 occurs primarily via the kidney, presumably by glomerular filtration. Thus, the single-chain recombinant mol. scFv-K12G0 is secreted in functionally intact form and it may be useful for targeting of radioisotopes or plasminogen activators to blood clots in vivo.

=> brusselbach and kontermann
BRUSSELBACH IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s brusselbach and kontermann
L5 0 BRUSSELBACH AND KONTERMANN

=> s brusselbach and muller
L6 0 BRUSSELBACH AND MULLER

=> s brusselbach
L7 0 BRUSSELBACH

=> dhis
DHIS IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> display his
ENTER (BRIEF), FULL, OR NOFILE:nofile
ENTER (L1-), L#, OR ?:L#

(FILE 'CAPLUS, BIOENG, BIOTECHNO, BIOTECHDS, ESBIOBASE' ENTERED AT 15:41:21 ON 19 SEP 2006)
L7 0 SEA ABB=ON PLU=ON BRUSSELBACH